# PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN

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I hereby declare that the thesis entitled **"PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN"** submitted towards partial fulfillment of the requirements for the award of **MD IN IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Rajesh Kumar,** Professor & Head, Department of I.H.B.T., **Dr. Pooja Tandon**, Professor, Department of Obstetrics & Gynaecology. No part of this thesis has formed the basis for the award of any degree previously.


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*Dedicated*

*to*

*my beloved Mother*

*Leelamma T.*

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*"God chose what is low and despised in the world, even things that are not, to bring to nothing things that are, so that no human being might boast in the presence of God."*

*1 Corinthians 1:28-29 ESV (The Holy Bible)*

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***Dr. Jannet Mary John***

# LIST OF ABBREVIATIONS

- **RhIg** : Rh immunoglobulin
- **HDFN** : Hemolytic Disease of the Fetus and Newborn
- **RBC** : Red Blood Cell
- **ABO** : Blood Group ABO
- **Ig** : Immunoglobulin
- **RBCs** : Red Blood Cells
- **HPA -1a** : Human Platelet Antigen-1a
- **FMH** : Fetomaternal hemorrhage
- **IAT** : Indirect Antihumanglobulin Test
- **AABB** : Association for the Advancement of Blood & Biotherapies
- **PEG** : Polyethylene Glycol
- **DAT** : Direct Antiglobulin Test
- **AHG** : Anti-Human Globulin
- **ELISA** : Enzyme-Linked Immunosorbent Assay
- **MCA-PSV** : Middle Cerebral Artery Peak Systolic Velocity
- **EDTA** : Ethylenediamine tetraacetic acid
- **Rh** : Rhesus
- **OPD** : Outpatient Department
- **C3d** : Complement Component 3d
- **SD** : Standard Deviation
- **n** : Sample size
- **%** : Percentage
- **y** : Years
- **$\chi^2$** : Chi-square test statistic
- **p** : p-value
- **G1, G2, G3, G4, G5** : Gravida status (number of pregnancies)
- **BOH** : Bad Obstetric History
- **NA** : Not Applicable

# ABSTRACT

**Introduction:** Hemolytic disease of fetus and newborn (HDFN) occurs due to maternal alloimmunization against red cell antigens. All pregnant women, including those who test positive for the D antigen, are screened in developed nations universally, while in underdeveloped nations like India, there are no such regulations. All red cell antibodies, with the exception of those that are naturally present (anti-A and anti-B), are unforeseen and can be either auto or alloantibodies. These antibodies may pass through the placenta during pregnancy and result in HDFN. Timely care of HDFN in pregnant women requires early identification.

**Aim of Study:** To study the prevalence of clinically significant red cell alloantibodies in pregnant females.

**Materials & methods:** A prospective study was carried out on 119 pregnant women attending antenatal clinics to detect the prevalence of clinically significant red cell alloantibodies. These women were typed for ABO-Rh antigens. Column agglutination technology was used for screening and identification for alloantibodies with the help of a 3-cell and 11-cell panel respectively. Once identified, titration was carried out. DAT and autocontrol was also performed in each case. Medical records and detailed obstetric history of these women were reviewed.

**Results:** The overall prevalence of alloantibodies in this study was 5.9% of this, 5% was constituted by anti-D. Alloimmunization rate in RhD negative patients was 11.7%. None of the RhD positive patients were alloimmunized.

**Conclusion:** Anti-D was the most common culprit responsible for alloimmunisation. Other alloantibody found included anti-D+C. Extensive population based research is necessary to determine the true extent of alloimmunization among Indian pregnant women. RhD positive women should have access to screening options especially in centers with capabilities for managing an allosensitized pregnancy.



**Keywords:** HDFN, alloimmunization, pregnant women, screening

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# INTRODUCTION

Even after RhD prophylaxis was first introduced more than 60 years ago, red cell sensitization during pregnancy continues to be a persistent difficulty for obstetricians and blood transfusion specialists. Prior to the availability of Rh immunoglobulin (RhIG), maternal anti-D alloimmunization was the leading cause of Hemolytic Disease of the Fetus and Newborn (HDFN) related to RhD incompatibility. The introduction of RhIG raised hopes that the occurrence of HDFN due to D antigen mismatch would decline. Over the years, various hypotheses have been proposed and extensively studied regarding the mechanisms of action of RhIG, yet the precise mechanism remains elusive.[1]

Even today, the challenge of alloimmunization during pregnancy persists and requires concerted efforts for resolution. Beyond the RhD antigen, other Rh system antigens (C, c, E, e, $C^w$) and various blood group systems have gained attention. Research on alloimmunization during pregnancy has been extensively conducted across different global regions. The antenatal immunohematological assessment of pregnant women involves screening for unexpected red blood cell antibodies in their blood during pregnancy. A positive result necessitates further investigation into the antibody's specificity, clinical significance, and its potential to move across the placental barrier and induce HDFN. [2]

HDFN is a serious complication during pregnancy, occurring when there is a difference in antigens between maternal and fetal RBCs. Different people produce antibodies differently in response to the same antigen due to intricate genetic factors that are still poorly understood. Clinically significant red cell alloantibodies are those capable of causing hemolysis of RBCs that carry the corresponding antigen. Maternal antibodies that can lead to HDFN include ABO antibodies or non-ABO alloantibodies,

which may be formed in response to exposure from a blood transfusion or previous pregnancy involving RBC antigen differences. [3]

The severity of HDFN is influenced by the class and subclass of maternal antibodies involved. Among various types of immunoglobulins (IgG, IgM, IgA, IgE, and IgD), only IgG crosses the placenta actively. This begins after 12 weeks of pregnancy and continues until the delivery. These antibodies traverse the placenta and can cause hemolysis, leading to fetal or neonatal complications such as anemia, hyperbilirubinemia, and potentially fatal hydrops fetalis. Untreated HDFN can result in fetal death. When these maternal antibodies bind to particular antigens on fetal red blood cells, hemolysis happens. The fetal spleen macrophages remove the antibody-coated cells from circulation. The quantity of antigenic sites on the fetal RBCs and the antibody concentration determine how quickly RBCs are destroyed.

HDFN is primarily caused by antibodies targeting antigens such as anti-D, followed by anti-K, anti-c, anti-E, and other Rh antibodies. Occasionally, antibodies against Fy (Duffy) or Jk (Kidd) antigens may also contribute. Currently, there is no immunoprophylaxis available to prevent non-D alloimmunization. However, research has explored the clinical potential of using anti-KEL and anti-HPA-1a immunoglobulins in animal models.

According to British Committee for Standards in Haematology guidelines, all pregnant women should undergo ABO and RhD antigen typing and screening for red cell antibodies early in pregnancy and again at 28 weeks gestation. In the Netherlands, mandatory screening for irregular antibodies is required for all pregnant women during the first trimester. In most of the developed countries, universal screening for irregular erythrocyte antibodies is compulsory for all pregnant women, including those who are D antigen-positive. However, developing countries lack standardized

guidelines for this screening. Additionally, published data indicates significant variation in alloimmunization rates across different geographic regions. It is widely accepted that evidence-based guidelines for screening alloantibodies in pregnant women are essential in developing countries like India to ensure proper childbirth management. [4]

Pregnant women who have been identified with RBC alloimmunization or have a history of HDFN require careful monitoring of their antibody levels. Throughout the pregnancy, continuous monitoring of the fetus is essential to assess fetal well-being, detect fetal anemia, and determine the optimal timing for delivery.

# AIM AND OBJECTIVE

To study the prevalence of clinically significant red cell alloantibodies in pregnant females.

# REVIEW OF LITERATURE

Before Rh immune globulin (RhIG) was introduced in 1968, hemolytic disease of the fetus and newborn (HDFN) was mainly caused by antibodies formed in the mother against the RhD. RhIG drastically reduced RhD-related HDFN cases. However, HDFN can still occur due to other RBC antigens like ABO blood group incompatibilities as well as other less common RBC antigen specificities. Even while RhD is still important, other RBC antibody specificities have either equaled or exceeded its incidence, which reflects how causes of HDFN have changed since the RhIG era. [5,6]

## Etiology

Clinical observations of fetal and newborn hemolytic disease trace back to the 17th century. In 1939, Levine and Stetson highlighted a case where a postpartum woman experienced a transfusion reaction after receiving her husband's blood, hypothesizing that she had developed antibodies which were against the father's antigen probably during fetomaternal hemorrhage (FMH). Later on, this antigen was discovered to be RhD, marking a critical advancement in understanding HDFN's immunological basis.

HDFN is caused by maternal alloantibodies that move across the placenta and destroy fetal RBCs. Unlike IgG, IgA and IgM antibodies do not cross the placenta, limiting their involvement in HDFN. [7]

Maternal antibodies target blood group antigens on fetal red blood cells inherited from the father, leading to their destruction.

**Pathogenesis of HDFN Caused by RBC Alloimmunization**

There exist multiple factors that impact both the severity of HDFN and maternal RBC alloimmunization. These include:

1. Antigenic exposure: Exposure of the maternal immune system to fetal RBC antigens during pregnancy or childbirth can trigger antibody production.
2. Maternal immune system factors: Individual variations in maternal immune response, including genetic predisposition and immune competence, affect the likelihood and intensity of alloimmunization.
3. Antibody specificity: The specificity of antibodies produced by the mother against fetal RBC antigens determines the severity of HDFN. Some antibodies are more likely to cause severe hemolysis than others.
4. Influence of ABO group: Maternal and fetal ABO blood group incompatibilities can also lead to HDFN, although typically less severe compared to RhD incompatibility.

These factors collectively determine the risk and severity of HDFN in pregnancies where maternal alloimmunization occurs.

**Diagnosis of HDFN Caused by RBC Alloimmunization**

It takes good coordination between the pregnant patient, her healthcare practitioner, her spouse, and the clinical laboratory staff to diagnose HDFN caused by previous RBC exposure and alloimmunization. [8]

It is advised that the type and antibody detection test should be performed during the first prenatal appointment, preferably in the first trimester of pregnancy. [9]

It is essential to obtain the pregnant woman's history at the initial prenatal appointment in order to determine whether the mother has a history of HDFN, past pregnancy outcomes, or a history of transfusions. A comparable risk in the current pregnancy may be indicated by severe illness and unfavorable results in previous pregnancies.

In the first affected pregnancy, sequential antibody titers are useful for evaluating intrauterine fetal anemia; however, in future pregnancies, their predictive value decreases.

**ABO, RhD, and Antibody Screen**

It is crucial to type for ABO and RhD and use the indirect antihuman globulin test (IAT) for antibody detection when doing prenatal HDFN testing. This test finds the anti-globulin phase reactive and 37°C reactive IgG alloantibodies which are clinically relevant. Sensitivity is increased by using two distinct screening RBC reagents that express widely distributed antigens, ideally homozygous. Prior to initiation of RhIG therapy in RhD negative patients, repeat testing is advised if the initial antibody screen is negative, particularly if there is a history of transfusion or unexpected antibodies. [10] Of the general population, only a small percentage (0.8–2%) has detectable RBC alloantibodies. [11]

While not mandated by AABB, routine prenatal examinations for pregnant patients should typically include an antibody screen. This screen assesses the risk of HDFN and determines if the mother should receive RhIg prophylaxis. [12]

The group O persons who have been typed for the most common and clinically significant RBC antigens provide the RBC reagents utilized in antibody screen tests. O group cells are chosen to prevent interference from anti-A and anti-B antibodies in detecting antibodies to other blood group systems. Patient antibody screening employs a specific set of screen cells manufactured with either two (R1R1 and R2R2) or three (R1R1, R2R2, rr) cells, each with a unique antigen mix.

A pooled screening reagent including RBCs from at least two separate people can be used for blood donor antibody screening. However, using pooled reagents for patient antibody screening is not acceptable. Because the pooled reagent RBCs contains several RBC populations, only one of the RBCs in the pool may express the target antigen, hence mixed-field agglutination in reactions involving these RBCs needs to be closely watched. Each reagent set or pooled reagent must include a minimum of one cell that tests positive for each of the following antigens: D, C, c, E, e, K, k, Fya, Fyb, Jka, Jkb, Lea, Leb, P1, M, N, S, and s. Additional antigens will also be present. Each package of screen cells or pooled reagent will come with a particular antigen profile sheet that lists the presence of known antigens. These profiles are unique to each lot and should not be swapped interchangeably. Ideally, the screen cell set or pooled reagent should include cells with homozygous expression of many antigens. Homozygous expression means the cell inherited only one allele at a genetic locus, resulting in a "double dose" of that antigen on the cell surface. In contrast, cells with heterozygous antigen expression inherit two different alleles at a genetic locus, causing the alleles to "share" antigen sites on the cell surface.

**Antibody Identification**

After detecting an antibody in the initial antibody screen, further testing and review are required to identify the antibody and assess its clinical significance. This usually entail using an antibody identification panel and going over the patient's medical history. Additionally, an auto control or Direct Antiglobulin Test (DAT), if not already conducted, is part of the initial follow-up testing/review. The methods and test phases used in the initial antibody detection testing should match those used in the antibody identification panel testing. Depending on the results of these initial tests, further additional testing may be necessary.

If the antibody screen is reactive during prenatal testing for HDFN, determining the antibody specificity is crucial. Since certain antibodies don't produce HDFN, example: cold-reactive IgM antibodies including anti-I, anti-IH, anti-$Le^a$, anti-$Le^b$, and anti-P1; they are usually ignored. Lewis system antibodies, although common in pregnant women, are also not associated with HDFN. IgM, IgG, or a mix of these antibodies, such as those against anti-M and anti-N, may result in mild to moderate HDFN.

To differentiate between IgM and IgG antibodies, serum can be treated with a sulfhydryl reagent like dithiothreitol or 2-mercaptoethanol, affecting IgM but not IgG reactivity. Rh-negative pregnant females may show weakly reactive anti-D antibodies, often due to RhIG administration. This passive anti-D persists for months and needs differentiation from active immunization, where a titer above 4 suggests active immunization.

When testing samples from pregnant patients, using PEG (polyethylene glycol) appears to minimize false positive results as compared to gel card and solid-phase methods. Verification of RhIG administration history is crucial, as serological testing alone may not distinguish between passive and active antibodies effectively.

An antibody identification panel consists of 11 to 20 blood group O reagent RBCs with varying antigen expressions. The panel is designed to have a diverse pattern of antigen presentations, facilitating differentiation between different antibodies. It includes cells that express Rh, Duffy, Kidd, and MNSs antigens in homozygous forms. Every panel is accompanied by a profile sheet detailing the antigens present on each cell and providing columns to record reactions. Additionally, uncommon cells that are positive for less frequent antigens or negative for very widespread antigens may be indicated by this profile sheet. Like screen cells, the profile sheet is specific to each lot and should not be exchanged with profiles from other panels.

## Direct Antiglobulin Test

The detection of antibodies bound to the RBCs is important in detecting HDFN. The Direct Antiglobulin Test (DAT) is employed to identify antibodies that coat RBCs in vivo. In the tube method, the patient's RBCs are thoroughly washed to remove any unbound antibodies. Following this, Anti-Human Globulin (AHG) reagent is added. If RBCs have either IgG antibodies or complement bound to their surface, they will agglutinate upon addition of AHG. If neither IgG antibodies nor complement is present on the RBCs, no agglutination will occur. To validate a negative result, Coombs' control cells are included in the test. The DAT can also be conducted using solid-phase adherence and gel methods in addition to the traditional tube method.

## Antibody Titers

After identifying an antibody, it is beneficial to measure its quantity or concentration, which can be achieved through antibody titration. For this purpose, other methodologies such as enzyme-linked immunoassay (ELISA), flow cytometry, or radioimmunoassay can also be utilized, albeit these may not be available in every laboratory. To conduct antibody titration, serum is diluted in a series of twofold steps and then tested against red blood cells (RBCs) that carry the specific antigen of interest. The titer is defined as the highest dilution where visible agglutination of 1+ or more is observed. Additionally, a numerical score can be assigned based on the intensity of the reaction. Each reaction is given a value, and the total score is calculated by summing these discrete values.

When conducting an antibody titer test, it's crucial to prepare dilutions meticulously to prevent errors. Cross-contamination from tubes with higher antibody concentrations can inaccurately elevate titer results. To mitigate this risk, use fresh pipette tips for each tube during dilution preparation and sort the data according to priority, interpreting the least diluted tube first.

Throughout the series of titer-level investigations, the phenotype of the RBCs chosen for use in titration testing should remain constant. All subsequent titer specimens should be tested against a homozygous cell if the initial titer was produced using a cell expressing the antigen in a homozygous manner. Additionally, the method of testing must remain constant. It has been noted that greater titer levels are produced by gel technique titers because they are more sensitive than those made by tube method titers. To create meaningful comparisons between samples, the technologist must make sure the test systems are as similar as feasible.[13] Titer-level studies are helpful in keeping an eye on pregnant patients with an IgG antibody that can result in HDFN. Pregnancy-related increases in antibody titer levels indicate that the fetus is antigen-positive and hence at risk of developing HDFN. A rising titer level can be a sign that more intrusive testing is required. In order to distinguish immunological anti-D from passively acquired anti-D as a result of RhIg injection, an antibody titer may also be utilized. Seldom has the titer level linked to RhIg injection more than 4. [14]

One method of verifying the existence of antibodies that were formerly referred as HTLA (high titer, low avidity) antibodies is to do a titer. Weakly positive reactions are obtained during the AHG phase of testing for these antibodies, which are directed against high-prevalence antigens. Even at large dilutions (up to 2,048), the weak responses continue to exist. [15]

The relative concentration of antibodies that can cross the placenta and cause HDFN must be determined by antibody titration. In order to determine the maximum dilution at which a response still happens, patient serum or plasma is serially diluted and tested against certain RBCs during the titration process. By determining the antibody's potency and possible danger of generating HDFN, this approach aids in directing decisions regarding clinical management and treatment. [16]

Patient serum is diluted and evaluated utilizing an indirect antiglobulin phase with anti-IgG reagent in antibody titration for HDFN testing. The reciprocal of the dilution or a titer score are used to express the titration's endpoint, which is the maximum dilution that exhibits a positive reaction. Clinical management decisions are guided by this approach, which quantifies the concentration of antibodies capable of producing HDFN.

Consistency is critical when performing antibody titration on patient serum for HDFN assessment. The method which is advised involves the saline antiglobulin tube test, where the serum is diluted and incubated with RBCs at 37°C for 60 minutes. Anti-IgG reagent is used in the indirect antiglobulin phase to detect IgG antibodies. While the saline tube method is standard, alternative methods have been proposed for antibody titration in clinical practice. [16,17]

For accurate antibody titration in HDFN assuming, it's crucial to use RBCs with consistent characteristics. Every titration should use RBCs with the same concentration, about the same storage time, and genotype (ideally homozygous for the target antigen). Since the titer test findings are hard to duplicate, the first serum or plasma samples should be frozen and used in parallel with subsequent tests to increase accuracy. A titer change is only deemed significant if it differs by more than two dilutions or if the score changes by more than ten.

For the right clinical correlation, the selected approach is essential. Comparative proficiency testing demonstrates that higher titers are obtained with methods that use gel columns or enhancing media. Therefore, if this approach is to be employed, the critical titer level for these other methods needs to be ascertained by the specific laboratory by reviewing the results of many pregnancies complicated by HDFN. [18]

16 is regarded as the critical titer for the suggested procedure. At roughly 18 to 20 weeks of gestation, a second titer should be performed if the first one was 16 or above. When a titer is consistently and reliably at ≥32, color Doppler imaging should be used to measure the middle cerebral artery peak systolic velocity (MCA-PSV) beyond 16 weeks of gestation. [19] For titers less than 32, repeat testing is advised every 4 weeks starting at 16 to 20 weeks gestation, and more frequently in the third trimester.

The severity of HDFN cannot be predicted only by antibody titer. The antibody titer may be somewhat elevated in certain sensitized pregnant women even as the fetus's infection worsens. Similarly, whether or not she is pregnant and whether the fetus is RhD-positive or RhD-negative, a previously sensitized woman can have a persistently high antibody titer. In some, the titer may rise quickly, indicating that HDFN severity may be getting worse. Except in the case of anti-K with a K-positive fetus, antibody titers that are consistently below the critical titer determined by the laboratory throughout pregnancy can be used to accurately predict an unaffected or mildly to moderately affected fetus. Titration studies conducted at the time of delivery are not advised as they offer no information that is therapeutically meaningful.

Monitoring and managing HDFN based on antibody titers help guide appropriate interventions to mitigate risks to the fetus during pregnancy.[20]

**Red Blood Cell Alloimmunization and HDFN in Israel**

Rahimi-Levene et al. (2020) conducted a comprehensive retrospective study in Israel, analyzing data from 90,948 women who gave birth in 2011, representing 70% of all births that year. According to the study, 1.0% of the women had alloantibodies discovered in their 5.8% positive antibody screen results. Anti-D was the most common alloantibody among these instances, detected in 39.3% of women who tested negative for D and had

a titer of 16 or higher. Other important antibodies, such as “anti-E, anti-K, and anti-c”, were found in addition to anti-D, either separately or in combination. The study shown that anti-D alloimmunization nevertheless caused substantial HDFN in 29% of affected fetuses and infants, even after anti-D prophylaxis was widely administered. 6.8% of fetuses with numerous maternal alloantibodies had severe HDFN, which in rare instances required intrauterine or neonatal exchange transfusions. This underscores the persistent risk of HDFN from non-D antibodies and the importance of comprehensive antibody screening and follow-up for D-negative women to mitigate risks and improve perinatal outcomes. [21]

**Alloimmunization in Pregnant Women in India**

A range of investigations conducted in India has shed light on notable findings regarding alloimmunization in pregnant women. One particular prospective study, which included 3,577 multigravida participants, reported an overall alloantibody prevalence of 1.25%. This study observed a significant difference between D antigen-negative women, who had a prevalence of 10.7%, and D antigen-positive women, who had a much lower prevalence of 0.12%. The majority of alloimmunization cases involved anti-D antibodies, which made up 78.4% of the total. This research underscored the heightened susceptibility of D antigen-negative women and stressed the importance of thorough, population-based studies to precisely measure alloimmunization rates and develop guidelines suited to the specific healthcare needs of the population. [22]

A further study that evaluated 5,347 pregnant women at a tertiary care hospital between January 2008 and January 2009 discovered an overall prevalence of allosensitization of 1.48%. Compared to Rh(D) positive women (0.08%), the rate was much greater in Rh(D) negative females (9.43%). The most frequent antibody in 8.85% of women who were Rh(D) negative was anti-D. This highlights the intricacy of alloimmunization

profiles and the necessity of rigorous screening methods in antenatal care to manage the risk of HDFN. [23]

A total of 1.1% of alloantibody prevalence was discovered in a “study including 2,060 multiparous pregnant women at the Government Maternity Hospital in Tirupati”. Rh D-antigen negative women had a substantially larger prevalence (12.8%) than D-antigen positive women (0.3%). The most common type of alloimmunization, accounting for 77.3% of cases, was anti-D antibody. Even with the use of prophylactic Rh-immunoglobulin, anti-D remained the predominate antibody, demonstrating its ongoing importance. According to the study, cost and infrastructure limitations limit the practicality of universal prenatal antibody screening in countries with limited resources, even though it may be desired. However, it was determined that focused screening was necessary for women with unfavorable obstetric histories. [24]

**Maternal Red Cell Alloimmunization in the USA and China**

A retrospective analysis of pregnant women who gave birth in the USA between May 1, 2017, and December 31, 2017, was carried out at Beaumont Hospital Royal Oak. Using electronic medical records to examine 4,548 women, the study discovered a 0.74% prevalence of clinically significant alloantibodies. Anti-E was the most frequently discovered major alloantibody, followed by anti-K. The findings underscored the importance of testing for maternal red cell antibodies at an early stage of pregnancy to facilitate the identification and management of high-risk pregnancies. Additionally, it emphasized the possible advantages of reducing the amount of incompatible red cell antigens that women of reproductive age are exposed to by using prudent transfusion procedures. [25]

An observational cohort study was carried out in China between January 2005 and December 2019 at two hospitals connected to Sun Yat-sen University. Maternal alloantibody profiles were used to classify 268 pregnant woman-fetus couples in order to measure maternal red cell alloimmunization. Important discoveries showed that anti-D antibodies, then anti-M antibodies, were the main causes of HDFN. A higher frequency of severe HDFN was linked to the presence of numerous antibodies. To reduce HDFN risks in impacted groups, the study recommended individualized care plans and thorough antibody screening procedures. [26]

**Comparative Analysis of Studies**

A comparative analysis of studies from different regions reveals several commonalities and differences. The higher rates of alloimmunization in Rh(D) negative women and the predominance of anti-D antibodies were consistent findings across studies. However, the studies also highlighted notable limitations, such as the lack of universal screening protocols in resource-constrained settings, variability in screening techniques, and the absence of longitudinal data to assess long-term outcomes.

In developed countries, universal screening for alloantibodies in antenatal women is a standard practice, significantly contributing to the management and reduction of HDFN incidence. In contrast, developing countries like India face challenges in implementing such protocols due to cost and infrastructure constraints. Studies in India emphasized the need for comprehensive, population-based studies to accurately gauge the extent of alloimmunization and the importance of targeted screening, especially for women with adverse obstetric histories.

## Conclusion

The reviewed studies underscore the critical importance of systematic antibody screening in antenatal care to effectively manage the risk of HDFN. Anti-D antibodies were consistently found to be the predominant cause of alloimmunization across different studies, highlighting the continued relevance of Rh-immunoglobulin prophylaxis. However, the presence of other clinically significant antibodies, particularly in non-Rh(D) positive women, underscored the complexity and varied immunological profiles among pregnant women.

The comparison between studies revealed several similarities, including the higher rates of alloimmunization in Rh(D) negative women and the predominance of anti-D antibodies. However, the studies also highlighted notable limitations, such as the lack of universal screening protocols in resource-constrained settings, variability in screening techniques, and the absence of longitudinal data to assess long-term outcomes.

Research gaps identified include the need for comprehensive, population-based studies to accurately gauge the extent of alloimmunization among pregnant women in developing countries, research on the cost-effectiveness of implementing universal screening protocols, and long-term studies to understand the implications of maternal alloimmunization on neonatal and maternal health outcomes. Addressing these gaps is essential to enhance maternal healthcare strategies and improve outcomes for both mothers and their infants.

# MATERIALS AND METHODS

The study includes all antenatal patients, irrespective of their period of gestation and obstetric history. Ethylenediamine tetraacetic acid (EDTA) sample will be used for ABO-Rh grouping and Gel vial sample for screening antibodies using polyspecific antihuman globulin gel cards (anti IgG+C3d) and a 3 cell and 11 cell panel (Diapanel, Bio-Rad, Switzerland). Once the antibody is identified, titration will be carried out. Direct Agglutination Test (DAT) and Auto control will be performed in each case to screen for autoantibodies. Adsorption and Elution methods will be used to further investigate for separation and identification of multiple antibodies present in the serum.

## SOURCE OF DATA

This study will be conducted among the pregnant females who are registered with the institute for antenatal checkups. OPD Records will be examined thoroughly for history.

The details will be filled as per the performa for each patient.

## METHOD OF COLLECTION OF DATA

**Inclusion Criterion:** All pregnant females irrespective of gestational age.

**Exclusion Criterion:** Previous history of blood transfusion, history of Rh immunization in present pregnancy, known case of any autoimmune disorders, on corticosteroid treatment.

**Duration of study:** 1 year.

**Statistical Analysis:** Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data is normally distributed Comparison of quantitative variables between the study groups will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-

parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and fisher exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows

# OBSERVATION AND RESULT

This study, entitled “prevalence of clinically significant red cell alloantibodies in pregnant women”, was conducted on 119 pregnant females who were registered with Dayanand Medical College and Hospital, Ludhiana for antenatal checkups from February 2023 to January 2024. The descriptive statistics are as follows:

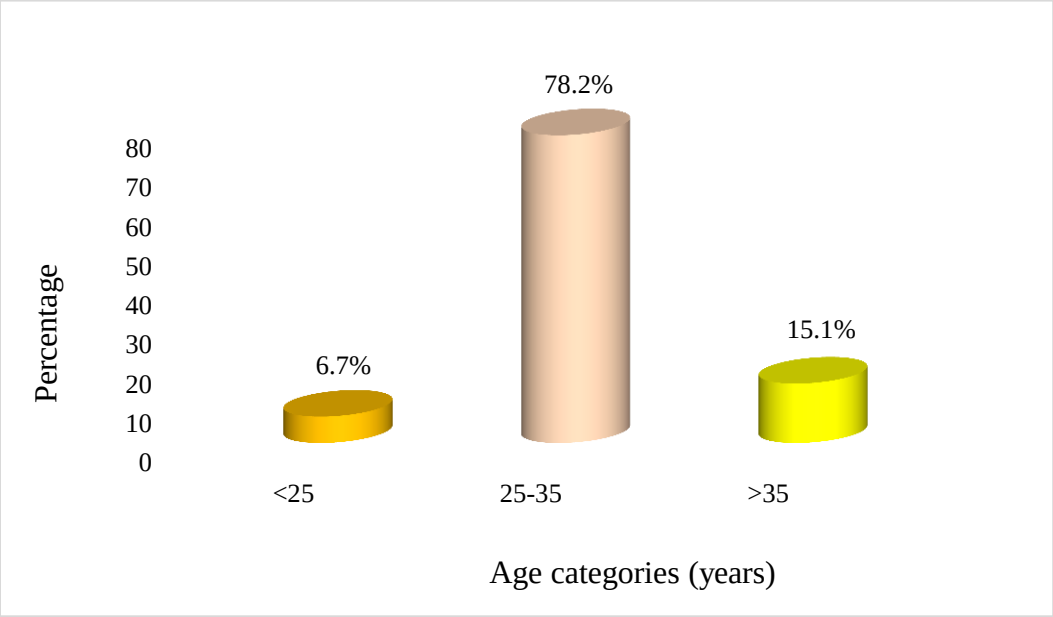


**Figure 1: Age wise distribution of patients**

The above figure shows that among 119 patients, majority 93(78.2%) were in the age category 25–35 years. This was followed by 18(15.1%) patients with age more than 35 years and least 8(6.7%) being those below 25 years of age. The Mean ± SD age of the patients was 30.64±4.56 years and ranged from 21 years to 45 years.

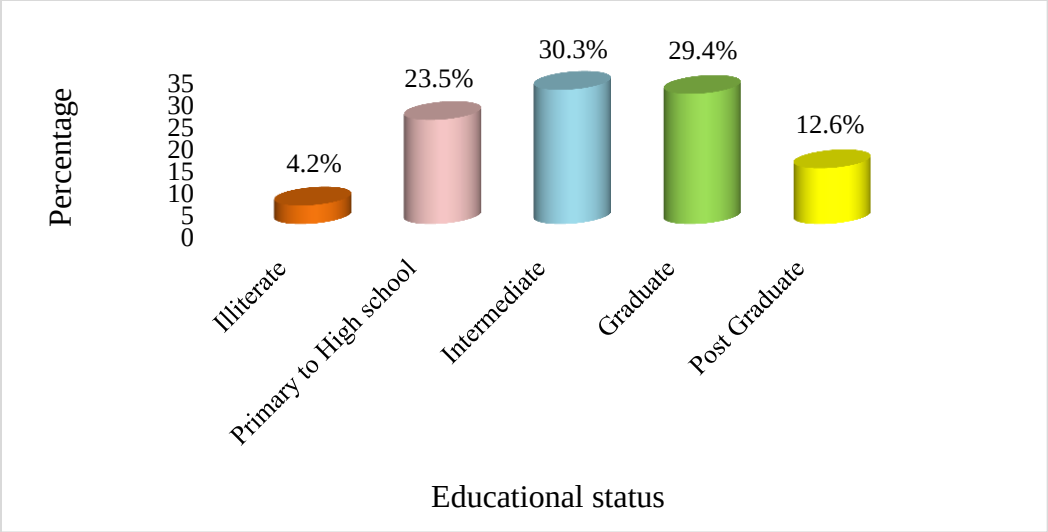


**Figure 2: Percentage of patients based on educational status**

As per the educational status of the patients, 5 (4.2%) were illiterate, 28 (23.5%) were primary to high school educated, 36 (30.3%) were intermediate, 35 (29.4%) were graduates and 15 (12.6%) postgraduates.

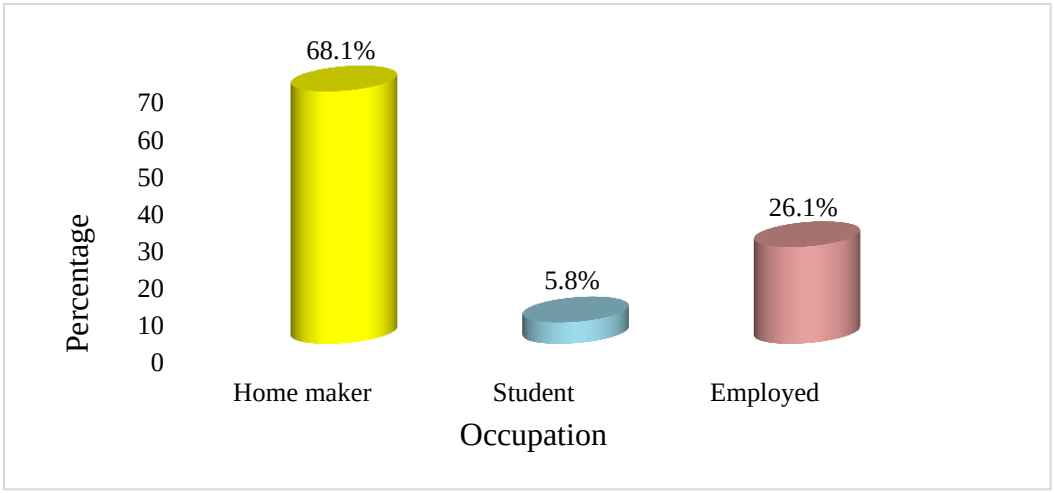


**Figure 3: Classification of patients according to their occupation**

In this study 81 (68.1%) were home maker, 31 (26.1%) were employed and 7 (5.9%) were students.

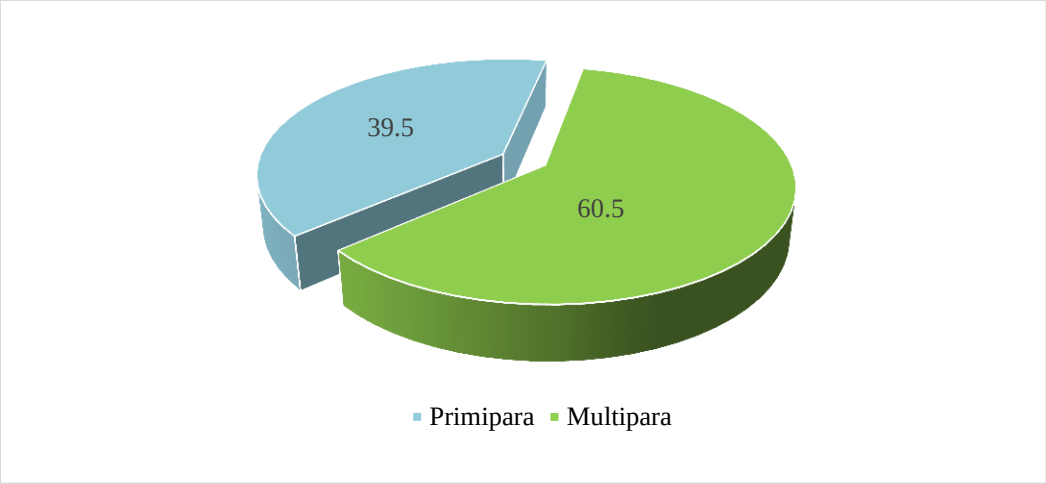


**Figure 4: Distribution of patients as per Parity status**

The findings regarding the parity status of the patients suggests that 72 (60.5%) were those who had crossed period of viability in at least one of the previous pregnancies (multipara), whereas 47(39.5%) were those who never had a pregnancy cross the period of viability (primipara).

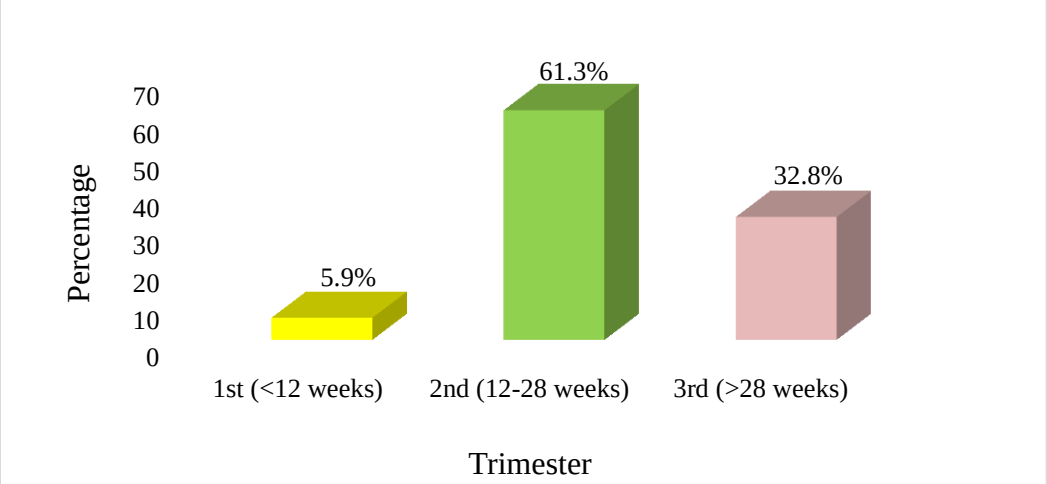


**Figure 5: Percentage of patients across various trimester**

The above data suggests that majority of the patients 73(61.3%) were in the second trimester of pregnancy, 39(32.8%) were in the third trimester while only 7(5.9%) patients were in the first trimester.

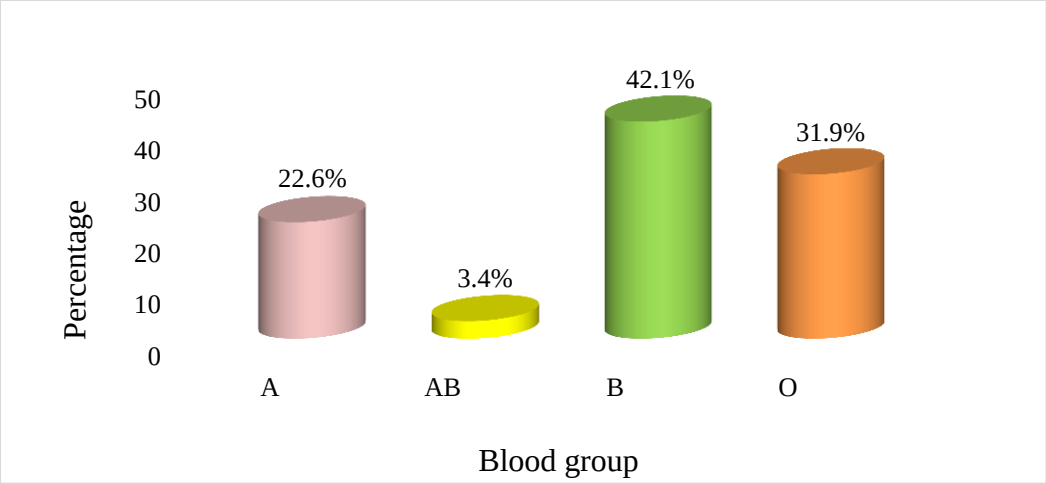


**Figure 6: Distribution of patients across ABO Blood groups**

Among the total 119 patients, majority 50 (42.1%) belonged to group B. This was followed by 38 (31.9%) group O, 27(22.6%) group A and least 4(3.4%) group AB.

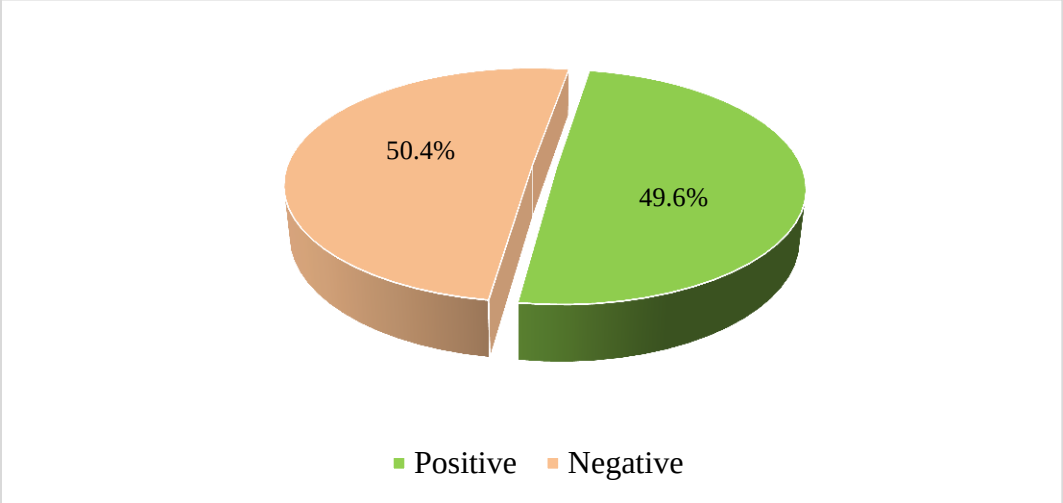


**Figure 7: Distribution of patients as per RhD status**

The above data illustrates that there were almost equal number of patients from both RhD positive blood group 59(49.6%) and RhD negative 60(50.4%).

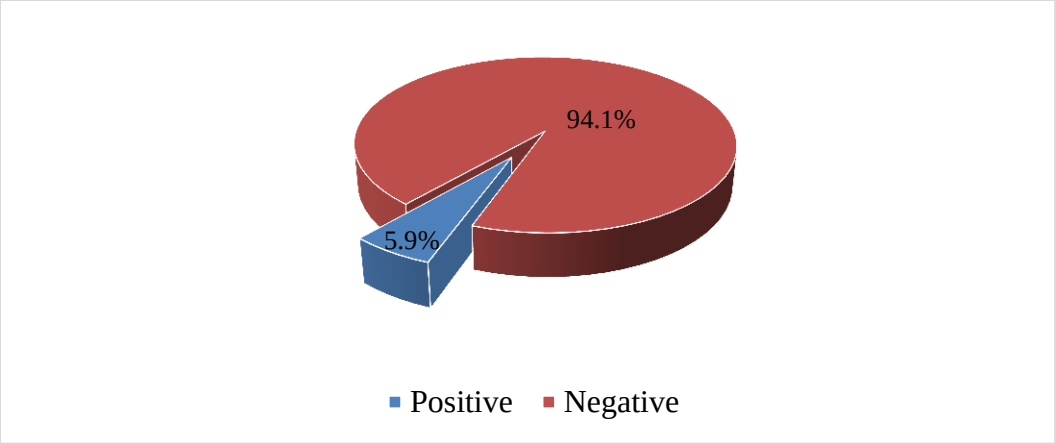


**Figure 8: Percentage of antibody screen positivity in patients**

Among all the patients (n=119), antibody screen was positive for 7 (5.9%) and negative for the majority 112 (94.1%).

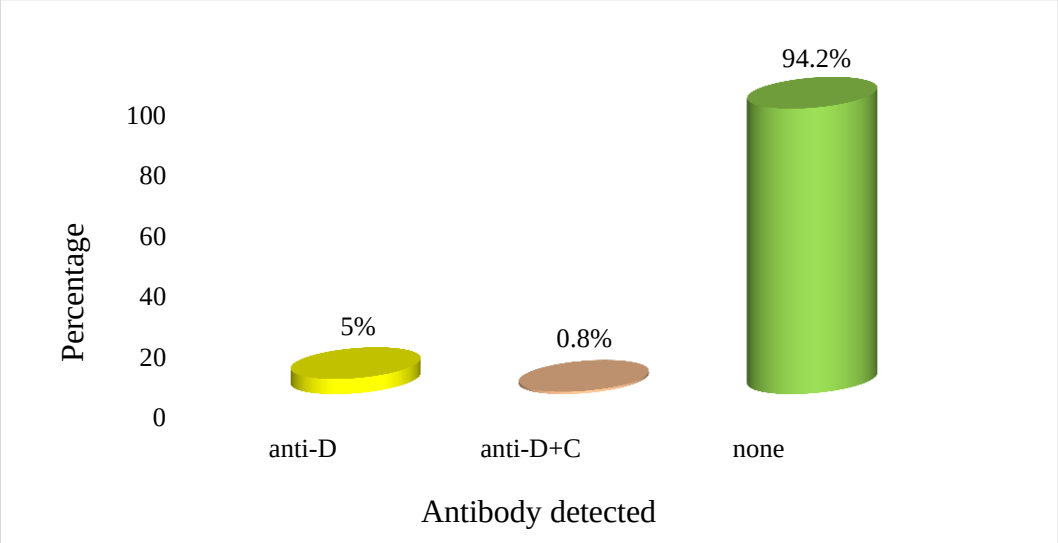


**Table 9: Frequency of alloantibodies detected**

In this study, 6(5%) patients had developed anti-D alloantibody whereas as 1(0.8%) developed anti-C in combination with anti-D.

**Table 1: Association of age with respect to alloimmunization**

| Age groups (years) | Antibody Screen | | $\chi^2$ | p |
|---|---|---|---|---|
| | Positive | Negative | | |
| | n(%) | n(%) | | |
| <25 | 0(0.0) | 8(100.0) | 1.432 | 0.489 |
| 25-35 | 5(5.4) | 88(94.6) | | |
| >35 | 2(11.1) | 16(88.9) | | |

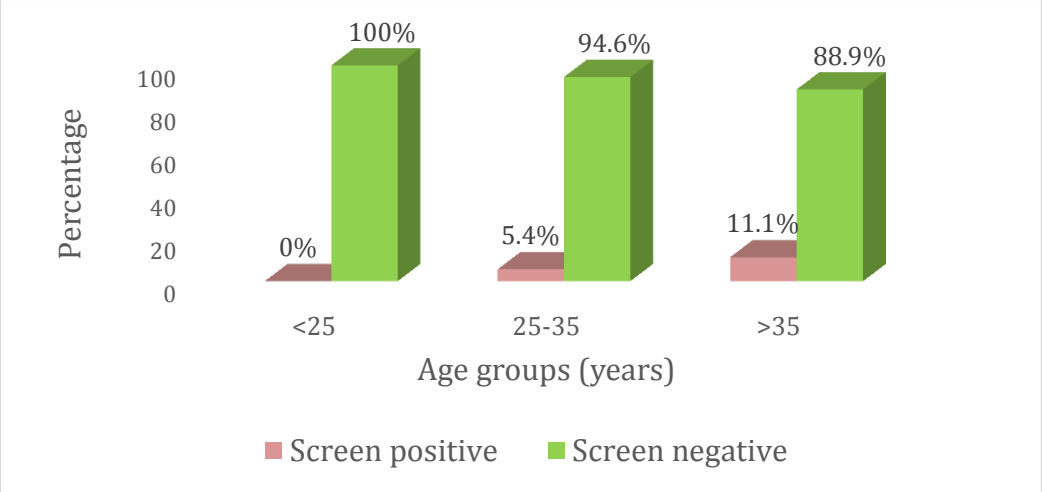


**Figure 10: Distribution of age with respect to alloimmunisation**

The above data depicts that there was no significant association between patient's age and alloimmunization ($p > 0.05$), though it is seen that alloimmunization was absent in patients below the age of 25 years as compared to 5(5.4%) in those between 25-35 years and 2(11.1%) in those above 35 years of age.

**Table 2: Association of alloimmunization according to Gravida status**

| Antibody screen | Gravida status | | | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G5 | | |
| | n (%) | n (%) | n (%) | n (%) | n (%) | | |
| Positive | 0 (0.0) | 4 (57.1) | 1 (14.3) | 1 (14.3) | 1 (14.3) | 5.92 | 0.205 |
| Negative | 45 (40.2) | 42 (37.5) | 14 (12.5) | 7 (6.3) | 4 (3.6) | | |

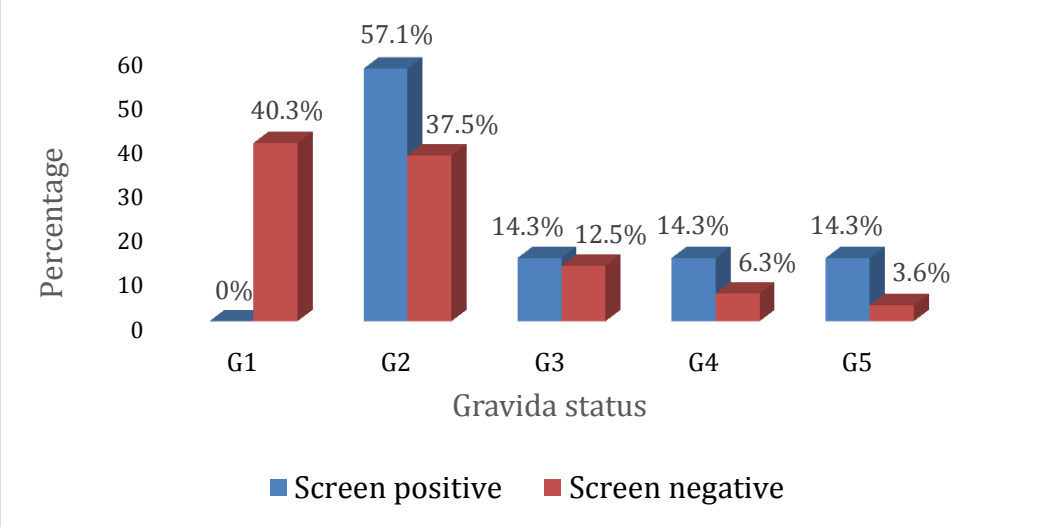


**Figure 11: Association of alloimmunisation according to Gravida status**

Among all the patients who showed alloimmunization (n=7), 4(57.1%) were in their second pregnancy, whereas 1(14.3%) patient each was pregnant for the third, fourth and fifth time. None of the patients in their first pregnancy showed any alloimmunization. This study did not show any significant association between gravida status and alloimmunization ($p > 0.05$).

**Table 3: Trimester wise alloimmunization**

| Trimester | Antibody Screen | | $\chi^2$ | p |
|---|---|---|---|---|
| | Positive | Negative | | |
| | n(%) | n(%) | | |
| 1st (<12 weeks) | 1 (14.3) | 6 (85.7) | 3.543 | 0.17 |
| 2nd (12-28 weeks) | 2 (2.7) | 71 (97.3) | | |
| 3rd (>28 weeks) | 4 (10.3) | 35 (89.7) | | |

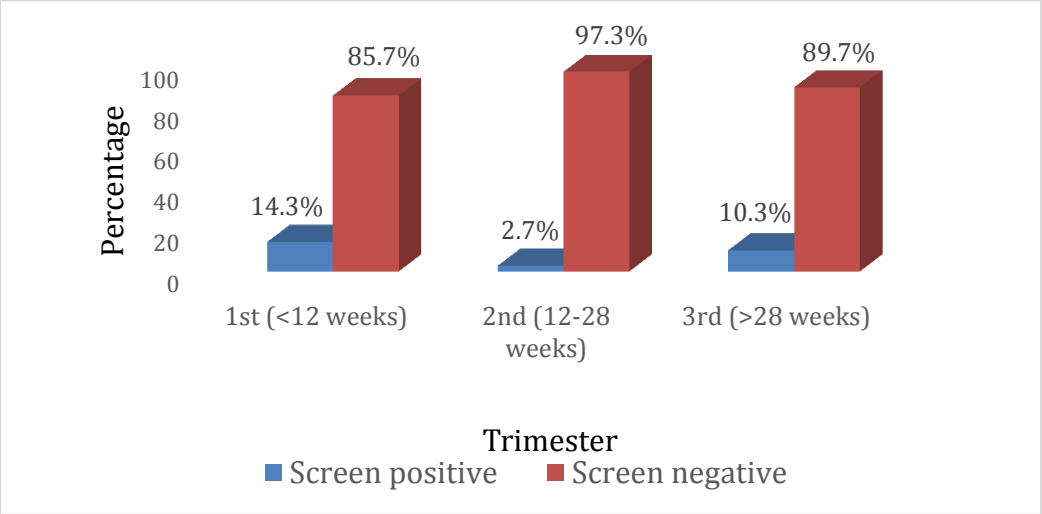


**Figure 12: Trimester wise alloimmunisation**

Among those patients in their third trimester (n=39), alloimmunization was present in 4(10.3%); whereas alloimmunization among those in their second (n=73) and first (n=7) trimester was 2(2.7%) and 1(14.3%) respectively. There was no significant association between the gestational age and alloimmunization ($p > 0.05$).

**Table 4: Association of bad obstetric history (BOH) with alloimmunization**

| BOH | Antibody Screen | | $\chi^2$ | p |
|---|---|---|---|---|
| | Positive | Negative | | |
| | n(%) | n(%) | | |
| Present | 1 (12.5) | 7 (87.5) | 0.678 | 0.41 |
| Absent | 6 (5.4) | 105 (94.6) | | |

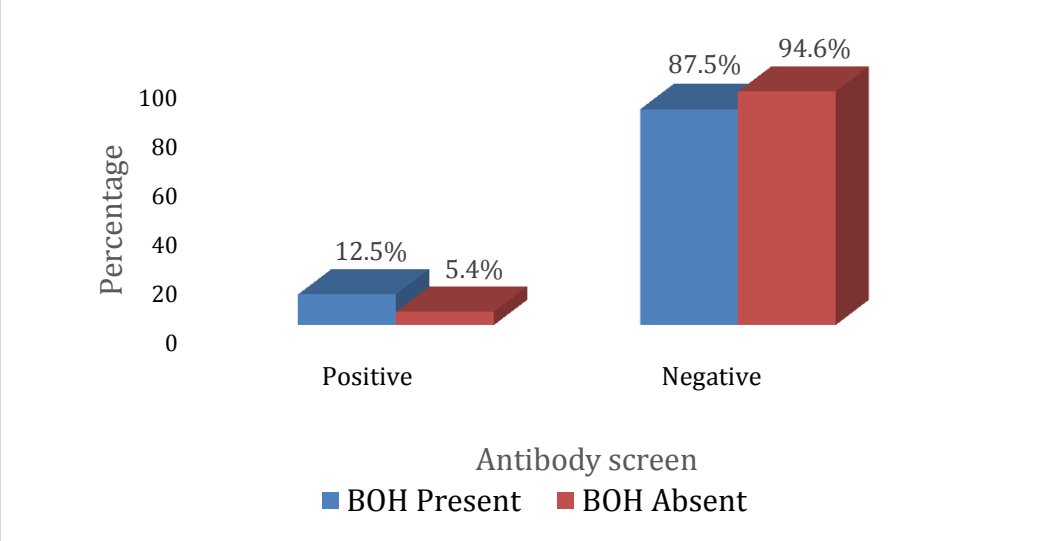


**Figure 13: Association of bad obstetric history (BOH) with alloimmunisation**

Alloimmunization was present in 1(12.5%) patient among those with bad obstetric history (n=8) and in 6(5.4%) among those with no bad obstetric history (n=111). This study did not show any significant association between bad obstetric history and alloimmunization ($p > 0.05$).

**Table 5: Alloimmunization across the ABO blood group**

| Antibody screen | ABO blood group | | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| | A | AB | B | O | | |
| | n (%) | n (%) | n (%) | n (%) | | |
| Positive | 1 (14.3) | 0 (0.0) | 4 (57.1) | 2(28.6) | 0.913 | 0.822 |
| Negative | 26(23.2) | 4(3.6) | 46(41.0) | 36(32.2) | | |

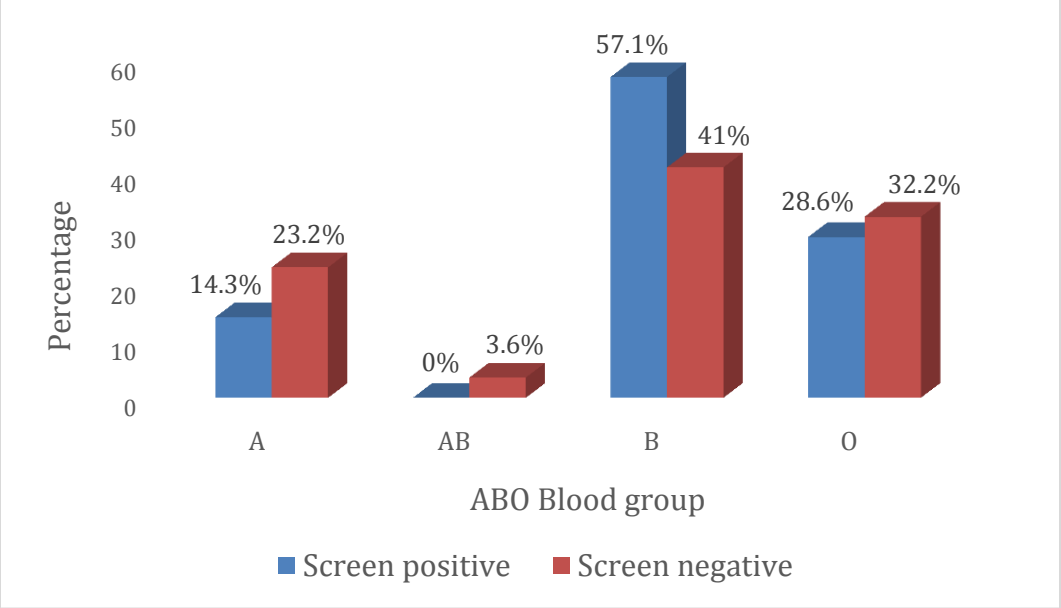


**Figure 14: Alloimmunisation across the ABO blood group**

According to the above data there is no significant association between ABO blood group and alloimmunization ($p > 0.05$). Among those with alloimmunization (n=7), 4(57.1%) patients belonged to group B, 2(28.6%) to group O and 1(14.3%) to group A. No alloimmunization was seen in group AB.

**Table 6: Association of RhD Antigen with alloimmunization**

| RhD | Antibody Screen | | $\chi^2$ | p |
|---|---|---|---|---|
| | Positive | Negative | | |
| | n(%) | n(%) | | |
| Positive | 0 (0.0) | 59 (100.0) | 7.314 | 0.007 |
| Negative | 7 (11.7) | 53 (88.3) | | |

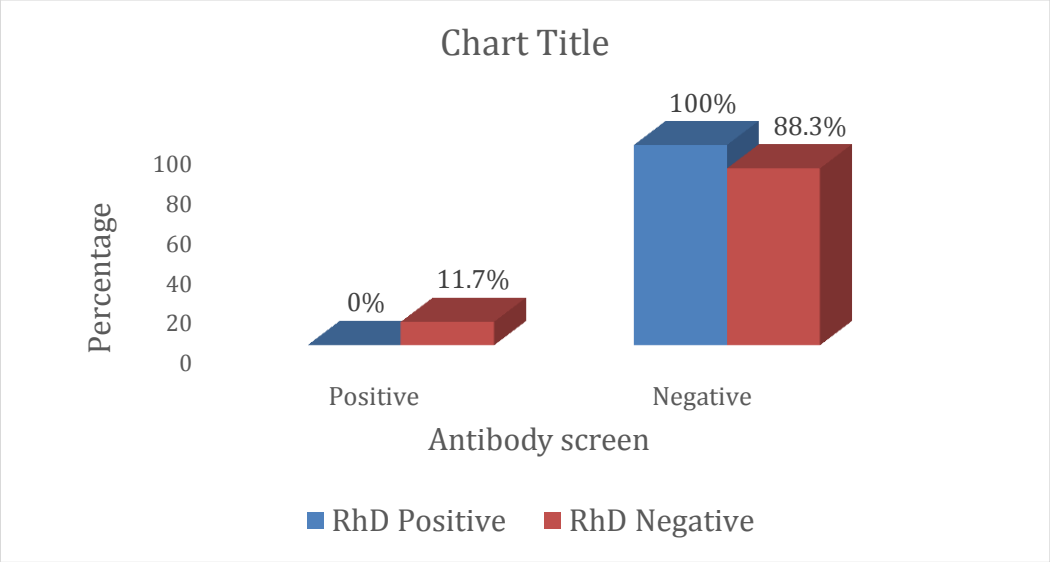


**Figure 15: Association of RhD Antigen with alloimmunization**

As per the data above, there is a significant association between alloimmunization and RhD status of the patient ($p < 0.05$). Alloantibodies were seen in 7(11.7%) RhD negative patients (n=60); whereas none of the RhD positive (n=59) patients were alloimmunized.

**Table 7: Association of RhD status with alloantibodies detected**

| RhD | Antibody | | $\chi^2$ | p |
|---|---|---|---|---|
| | anti-D | anti-D+C | | |
| | n (%) | n (%) | | |
| Positive | 0 (0.0) | 0 (0.0) | 7.314 | 0.026 |
| Negative | 6 (10.0) | 1 (1.7) | | |

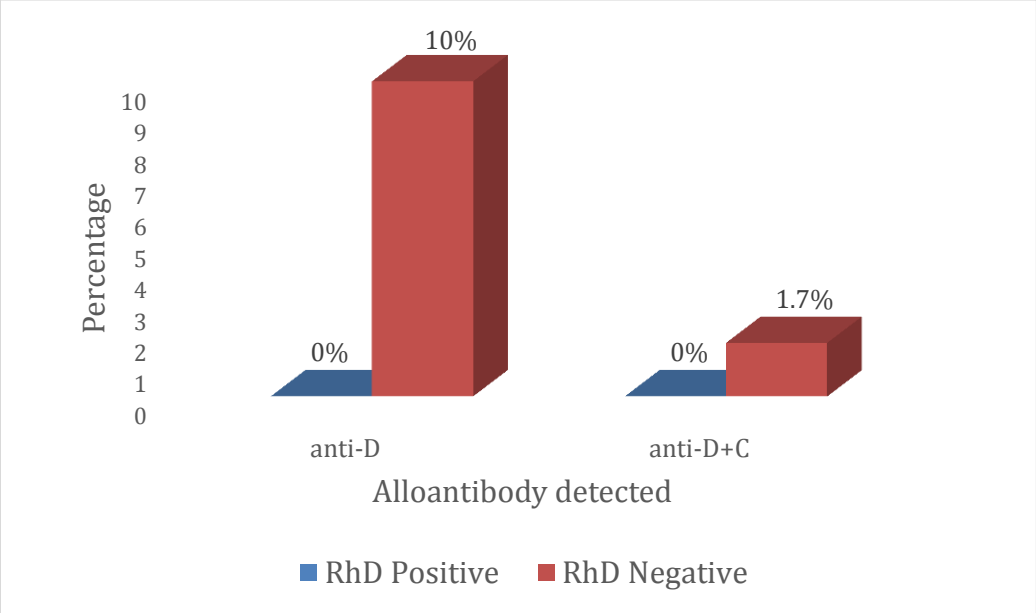


**Figure 16: Association of RhD status with alloantibodies detected**

Among the RhD negative patients (n=60), 6(10.0%) had anti-D alloantibody; whereas 1(1.7%) had a combination of anti-D and anti-C. There is a significant association between the alloantibodies detected and the RhD status of the patient ($p < 0.05$).

**Table 8: Distribution of alloantibodies detected across ABO blood group**

| Antibody detected | ABO group | | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| | A | AB | B | O | | |
| | n (%) | n (%) | n (%) | n (%) | | |
| anti-D | 1 (16.7) | 0 (0.0) | 3 (50.0) | 2 (33.3) | 1.826 | 0.935 |
| anti-D+C | 0 (0.0) | 0 (0.0) | 1 (100.0) | 0 (0.0) | | |

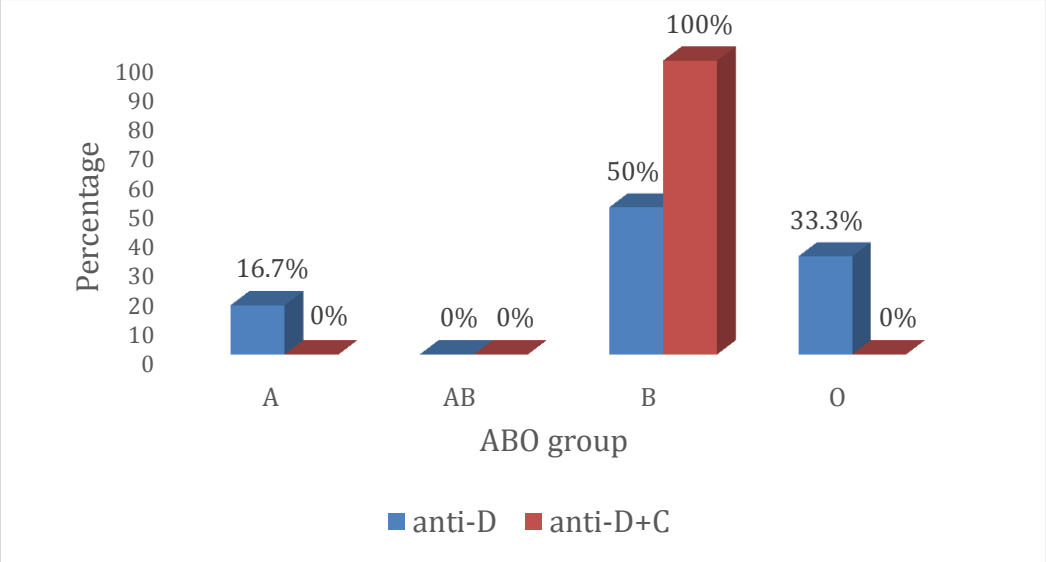


**Figure 17: Distribution of alloantibodies detected across ABO blood group**

Among the alloantibodies detected, anti-D (n=6) was present in 3 (50.0%) patients with blood group B, 2(33.3%) in those with blood group O and 1(16.7%) in blood group A. The combination of anti-D and anti-C (n=1) was seen only in the blood group B. There was no significant association seen between the ABO blood group and the alloantibodies detected (p>0.05).

**Table 9: Relationship of alloantibodies detected with respect to the titer score**

| Antibody detected | Antibody titers | | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| | 32 | 64 | 256 | 512 | | |
| | n (%) | n (%) | n (%) | n (%) | | |
| anti-D | 1 (16.7) | 2 (33.3) | 2 (33.3) | 1 (16.7) | 1.556 | 0.67 |
| anti-D+C | 0 (0.0) | 0 (0.0) | 1 (100.0) | 0 (0.0) | | |

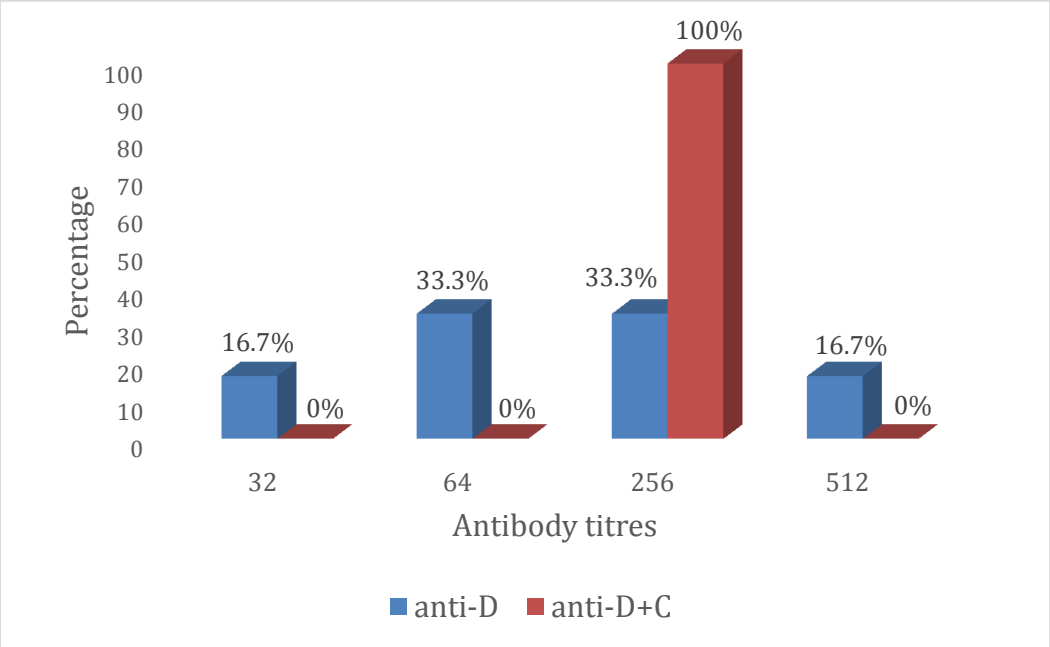


**Figure 18: Relationship of alloantibodies detected with respect to titer score**

The above data suggests no association between the alloantibodies detected and their titers (p>0.05). The titer of anti-D (n=6) was 256 and 64 in 2 (33.3%) patients each and 32 and 512 in 1(16.7%) patient each; whereas the titer of the combination of anti-D and anti-C (n=1) was 256.

# DISCUSSION

The present study was aimed to evaluate “the prevalence of clinically significant red cell alloantibodies in pregnant women”. During this study 119 pregnant females were screened and alloimmunization was found to be 5.90%. The comparison of the published world rates of alloimmunization in pregnancy is as shown below:

**Table 10: Comparison of prevalence of clinically significant red cell alloantibodies in pregnant women in various studies with present study**

| Authors | Place | Total no. of women screened | Study Year | Prevalence |
|---|---|---|---|---|
| Varghese et al [27] | Vellore | 5347 | 2008-09 | 1.48% |
| Pahuja S et al[28] | Delhi | 3577 | 2008-09 | 1.25% |
| Rahimi-Levene et al[29] | Israel | 90948 | 2011 | 5.80% |
| Suresh et al[30] | Tirupati | 2060 | 2012-13 | 1.10% |
| Dholakiya et al[31] | Gujarat | 8920 | 2014-16 | 1.31% |
| Moinuddin et al[32] | Southeast Michigan | 4548 | 2017 | 0.74% |
| **Present study** | **Ludhiana** | **119** | **2023-24** | **5.90%** |

The higher prevalence found in the present study could be attributed to multiple factors. Since only hospital attendees were included in this study, it is not indicative of the prevalence of alloimmunization among the vast

majority of pregnant Indian women, who lack access to obstetric care at a tertiary private centre. Also, 50.4% of the patients screened were RhD negative which is significantly higher as compared to other Indian studies with only 6-11%.[27-32] This also shows the bias in not screening RhD positive patients which is still a practice among the obstetricians in developing countries.

**BLOOD GROUPING DISTRIBUTION OF PATIENTS:**

In the present study, majority alloimmunisation was seen in blood group B. This could be attributed to the higher prevalence of blood group B in the North Indian population as stated in a study done by Mangwana et al.[33]

**Table 11: Comparison of clinically significant alloantibodies in various ABO blood groups of a similar study with present study**

| Authors | A % | B % | O % | AB % |
|---|---|---|---|---|
| Moinuddin et al[32] | 47.1 | 14.7 | 29.4 | 8.8 |
| **Present study** | **14.3** | **57.1** | **28.6** | **0.0** |

In a similar study from southeast Michigan, most alloantibodies were seen in Blood group A, followed by group O. [32]

**RHD STATUS OF PATIENTS:**

The alloimmunisation rate seen in RhD negative patients was 11.7% in this study; this finding is in accordance with previous literature (9.43%, 10.4% and 12.8%).[27,28,30] This portrays the Indian scenario, despite the introduction of RhIg.

Contradicting to the above literature, Moinuddin et al found that the rate of alloimmunization among RhD negative patients was 1.10%, which could be attributed to better access to health care services and proper adoption of immunoprophylaxis in the developed countries. [32]

**Table 12: Comparison between various studies for alloimmunisation with respect to RhD status**

| Authors | % RhD positive | % RhD negative |
|---|---|---|
| Varghese et al[27] | 0.08 | 9.43 |
| Pahuja S et al[28] | 0.12 | 10.40 |
| Suresh et al[30] | 0.30 | 12.80 |
| Dholakiya et al[31] | 0.38 | 19.45 |
| Moinuddin et al[32] | 0.70 | 1.10 |
| **Present study** | **0.00** | **11.70** |

Due to the low rate of allosensitization in women who are RhD positive, a routine screening program might not be practical. Nonetheless, it is proposed that RhD positive women should have access to screening options in centers with capabilities for managing an allosensitized pregnancy.

**GRAVIDA STATUS OF THE PATIENTS:**

The current study showed an increase in prevalence of alloimmunisation as the gravida status increased, maximum being found in fifth pregnancy. Similar trends have been seen in various studies where maximum alloimmunisation is found in fourth and fifth pregnancy. [28,30,31] Fetomaternal hemorrhage is the leading cause of maternal alloimmunization and with each pregnancy the chances of maternal antigenic exposure increases.

**Table 13: Comparison of alloimmunisation in relation to gravida status in various studies**

| Authors | % G1 | % G2 | % G3 | % G4 | % G5 | %G6 | %G7 |
|---|---|---|---|---|---|---|---|
| Pahuja S et al[28] | NA | 0.94 | 1.47 | 4.00 | 7.14 | NA | NA |
| Suresh et al[30] | NA | 0.80 | 1.30 | 10.50 | 0.00 | 0.00 | 0.00 |
| Dholakiya et al[31] | NA | 0.98 | 1.24 | 4.83 | 4.17 | NA | NA |
| **Present study** | **0.00** | **8.70** | **6.67** | **12.50** | **20.00** | **NA** | **NA** |

Slight variation in this trend can be attributed to the difference in the number of patients found in each gravida category.

**ALLOIMMUNISATION IN PATIENTS WITH RESPECT TO BAD OBSTETRIC HISTORY:**

One among the 8 patients having bad obstetric history (BOH) showed alloimmunisation in the present study. The other 6 showing alloimmunisation did not have any bad obstetric history. In two other studies from Delhi[28] and Gujrat[31], the prevalence of alloimmunisation among patients with BOH was found to be 5.50% and 5.00%, whereas among those with no BOH was 0.52% and 0.39% respectively. A similar finding was seen in another report where the prevalence among those with BOH was 1.92% and those without BOH was 0.94%.[30]

**Table 14: Comparison of various studies based on alloimmunisation with respect to bad obstetric history**

| Authors | BOH present (%) | BOH absent (%) |
|---|---|---|
| Pahuja S et al[28] | 5.50 | 0.52 |
| Suresh et al[30] | 1.92 | 0.94 |
| Dholakiya et al[31] | 5.00 | 0.39 |
| **Present study** | **12.50** | **5.40** |

The prevalence seen is higher than that in the other studies which could be attributed to the higher overall prevalence of alloimmunisation in our current study. Nevertheless, the association between the presence of BOH in pregnant women and alloimmunisation is established in all of these studies.

**ALLOANTIBODIES DETECTED IN THE PATIENTS:**

In this study the alloantibodies identified were anti-D (85.7%) and anti-D+C (14.3%). No other alloantibodies were found. A similar report showed anti-D (75.6%) and anti-D+C (13.3%), but also alloantibodies like anti-M (4.4%), anti-K (2.2%) and others (4.5%)[28]. Another study detected anti-D (63.6%) and anti-D+C (13.7%), also anti-E, anti-$Le^a$ and anti-M (4.5% each). [30] In yet another study, alloantibodies other than anti-D were constituted by anti-M (10.3%), anti-E (7.1%) and others (20.7%).[31] In a study conducted by Varghese et al to characterize the clinically significant alloantibodies in pregnant women, 34.2% were anti-D whereas anti-$Le^a$ (10.1%), anti-D+C (3.8%) and anti-M (1.3%) were among those that were identified. [27]

Segregation was done based on single and multiple alloantibodies identified in a large study published from Israel, in which most frequent single alloantibody was anti-E (17.7%) followed by anti-K (14.8%), anti-$Le^a$ (12.1%) and anti-M (11.2%). Among multiple alloantibodies identified, anti-c+E constituted majority (30.4%), followed by anti-$Le^a$+$Le^b$ (12.5%) and anti-D+C (7.1%).[29] A report from Southeast Michigan determined the prevalence and specificity of clinically significant maternal red cell alloantibodies to be mainly anti-E (38.2%), anti-K (20.6%) and anti-M (17.6%) along with few others. No anti-D was found in this study. [32]

**Table 15: Comparison of alloantibodies identified among pregnant women in various studies**

| Authors | % Anti-D | % Anti-D+C | % Anti-Le$^a$ | % Anti-E | % Anti-K | % Anti-M | % Others | Comments |
|---|---|---|---|---|---|---|---|---|
| Varghese et al[27] | 34.2 | 3.8 | 10.1 | NA | NA | 1.3 | 50.6 | Anti-E+K (1.3%) |
| Pahuja S et al[28] | 75.6 | 13.3 | NA | NA | 2.2 | 4.4 | 4.5 | |
| Rahimi-Levene et al[29] | 8.2 | * | 12.1 | 17.7 | 14.8 | 11.2 | 36.0 | * prevalence of multiple antibodies calculated separately |
| Suresh et al[30] | 63.6 | 13.7 | 4.5 | 4.5 | NA | 4.5 | 9.2 | |
| Dholakiya et al[31] | 61.9 | NA | NA | 7.1 | NA | 10.3 | 20.7 | Anti-C+e (7.1%) |
| Moinuddin et al[32] | NA | 2.9 | NA | 38.2 | 20.6 | 17.6 | 20.7 | |
| **Present study** | **85.7** | **14.3** | **NA** | **NA** | **NA** | **NA** | **NA** | |

# SUMMARY AND CONCLUSION

In this study, researcher investigated the prevalence of clinically significant red cell alloantibodies among 119 pregnant women. The findings revealed an overall alloimmunization rate of 5.9%. This indicated that a significant proportion of pregnant women in the study population have developed antibodies against red blood cells, highlighting the importance of monitoring and managing alloimmunization during pregnancy to ensure maternal and fetal health.

These results underscore the need for continued surveillance and proactive interventions to address potential complications associated with red cell alloimmunization in pregnancy.

Of the total patients, 60.5% were multiparous and highest alloimmunization (20.0%) was seen among those pregnant for the fifth time. Majority of the patients screened were in the second trimester (61.3%) of their pregnancy. 57.1% of those alloimmunized belonged to the blood group B, which was also the most prevalent ABO blood group (42.1%). Almost equal numbers of patients were screened from both RhD positive (49.6%) and RhD negative (50.4%) blood group; with 11.7% prevalence of alloimmunization within RhD negative group. Alloimmunization was detected in 12.5% of patients having bad obstetric history, whereas only 5.4% among those without any bad obstetric history. Of the alloantibodies detected, 5% was constituted by anti-D and 0.9% by combination of anti-D+C. The screening was done just once, regardless of the gestational age. Therefore, it is possible that some antibodies would have been present at a later stage of pregnancy and thus missed. The husband’s blood group could not be documented even in Rh-D negative women, making it difficult to determine the precise level of alloimmunization in Rh negative women. Also, increasing the sample size would enhance the study’s statistical power, allowing for more robust

conclusions and potentially identifying additional factors influencing alloimmunization rates.

Widespread prenatal antibody screening though ideal, may not be feasible in developing countries like India. The factors influencing this could be challenges with infrastructure, high costs and investments, limited awareness among patients and medical fraternity, improper government policies and regulations. Despite this, it is advised to implement carefully thought-out processes even in resource poor settings, to screen at least the expectant mothers with bad obstetric history. A routine screening program might not be practical in women who are RhD positive due to the low rate of allosensitization. Nonetheless, it is proposed that RhD positive women should have access to screening options in centers with capabilities for managing an allosensitized pregnancy.

The way forward would be to explore the barriers and the need to build capacity among medical professionals to provide the best care possible to the mother and baby.

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# KEY TO MASTER CHART

- ✓ **Edu.** : Education
- ✓ **Occ.** : Occupation
- ✓ **MF** : Married for
- ✓ **Y** : year
- ✓ **LMP** : Last menstrual period
- ✓ **EDD** : Expected due date
- ✓ **GA** : Gestational Age
- ✓ **Obs score** : Obstetric score
- ✓ **Primi** : Primigravida
- ✓ **GPAL (Obs score)** : Gravida Parity Abortion Living
- ✓ **IUD** : Intra uterine death
- ✓ **SB** : Still birth
- ✓ **NND** : Neonatal death
- ✓ **IVF** : In vitro fertilization
- ✓ **ART** : Anti retroviral therapy
- ✓ **GDM** : Gestational diabetes mellitus
- ✓ **COP** : Cholestasis of pregnancy
- ✓ **DM** : Diabetes mellitus
- ✓ **uGDM** : uncontrolled Gestational diabetes mellitus
- ✓ **Neg** : Negative
- ✓ **Pos** : Positive
- ✓ **DAT** : Direct antiglobulin test
- ✓ **AC** : Auto control
- ✓ **IAT** : Indirect antiglobulin test
- ✓ **Ab.** : Antibody
- ✓ **ID** : Identification

| S.NO. | Name | Age | MRD NO. | Edu. | Occ. | MF | LMP | EDD | GA | Obs Score | High Risk | ABO Group | DAT | AC | IAT | Ab. Screen | Ab. Titer | Ab. ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | BR | 39y | 7541 | Illiterate | House Wife | 20y | 30.07.22 | 12.08.23 | 26w2d | G2P1L1 | IVF | A+ | Neg | Neg | Neg | Neg | | |
| 2 | LR | 29y | 1393168 | BA | House Wife | 6y | 01.08.22 | 08.05.23 | 28w0d | G2P1L1 | none | A+ | Neg | Neg | Neg | Neg | | |
| 3 | RK | 37y | 1462248 | B. Ed | House Wife | 12y | 17.12.22 | 23.09.23 | 11w3d | G3P2 IUD2 | BOH, on ART | A neg | 1+ | Neg | Neg | Neg | | |
| 4 | MD | 31y | 1014584 | 10th | House Wife | 12y | 09.07.22 | 15.04.23 | 28w6d | G4P2L1A1SB1 | BOH | A+ | Neg | Neg | Neg | Neg | | |
| 5 | P | 24y | 1370677 | B. Ed | House Wife | 1y | 14.07.22 | 20.04.23 | 30w4d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 6 | A | 29y | 335955 | 12th | Staff Nurse | 2y | 20.12.22 | 26.09.23 | 11w6d | G2P1L1 | none | B+ | Neg | Neg | Neg | Neg | | |
| 7 | R | 25y | 1404261 | Illiterate | House Wife | 3y | 24.10.22 | 31.07.23 | 17w0d | G4A3 | BOH | O neg | Neg | Neg | Neg | Neg | | |
| 8 | M | 24y | 1150872 | M. Sc. | Student | 2y | 20.10.22 | 27.07.23 | 17w4d | Primi | none | A+ | Neg | Neg | Neg | Neg | | |
| 9 | KK | 26y | 1414302 | 12th | House Wife | 2y | 30.11.22 | 06.09.23 | 13w0d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 10 | BK | 32y | 1472463 | 12th | House Wife | 9y | 20.01.23 | 22.10.23 | 14w5d | G3P2L2 | none | A+ | Neg | Neg | Neg | Neg | | |
| 11 | LD | 29y | 707244 | M. Sc. | Staff Nurse | 1y | 10.11.22 | 17.08.23 | 15w6d | Primi | none | AB+ | Neg | Neg | Neg | Neg | | |
| 12 | SD | 24y | 1437621 | Illiterate | House Wife | 4y | 08.11.22 | 15.08.23 | 16w3d | G5P4IUD2NND2 | BOH | A+ | Neg | Neg | Neg | Neg | | |
| 13 | B | 29y | 315319 | 10th | Staff Nurse | 1y | 08.12.22 | 14.09.23 | 12w1d | Primi | GDM on Oral meds | B+ | Neg | Neg | Neg | Neg | | |
| 14 | N | 30y | 1475492 | B. Ed | House Wife | 1y | 04.07.22 | 10.04.23 | 35w0d | G2A1 | none | B+ | Neg | Neg | Neg | Neg | | |
| 15 | PS | 33y | 1463589 | 12th | Staff Nurse | 1y | 15.01.23 | 22.10.23 | 12w2d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 16 | MS | 27y | 1346609 | M. CS. | House Wife | 3y | 18.08.22 | 25.02.23 | 28w1d | Primi | Overt Hypothyroid | B+ | Neg | Neg | Neg | Neg | | |
| 17 | K | 26y | 1388338 | 12th | House Wife | 4y | 14.08.22 | 21.05.23 | 29w1d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 18 | SK | 30y | 438466 | B. Sc. | Staff Nurse | 1y | 09.01.23 | 16.10.23 | 18w4d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 19 | RK | 45y | 169208 | 10th | House Wife | 7y | 22.12.22 | 28.09.23 | 11w1d | G2P1L1 | none | B neg | Neg | Neg | 4+ | Pos | 64 | anti-D |
| 20 | B | 28y | 277434 | 12th | Staff Nurse | 1y | 26.10.22 | 02.08.23 | 19w2d | Primi | none | A+ | Neg | Neg | Neg | Neg | | |
| 21 | K | 32y | 8773 | B. PT. | Physiotherapist | 7y | 05.11.22 | 12.08.23 | 17w6d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 22 | K | 26y | 1076636 | 12th | House Wife | 4y | 20.01.23 | 27.10.23 | 11w2d | G2P1L1 | none | B neg | Neg | Neg | Neg | Neg | | |
| 23 | ZA | 24y | 1473795 | B.A. | House Wife | 2y | 09.10.22 | 16.07.23 | 21w5d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 24 | SK | 38y | 387876 | 12th | House Wife | 5y | 05.11.22 | 12.08.23 | 17w1d | G4P3L3 | none | B+ | Neg | Neg | Neg | Neg | | |
| 25 | A | 33y | 664175 | 7th | House Wife | 14y | 13.01.23 | 20.10.23 | 14w1d | G5P2L2A2 | BOH | A+ | Neg | Neg | Neg | Neg | | |
| 26 | A | 26y | 1315535 | B. A. | House Wife | 2y | 10.08.22 | 07.05.23 | 31w0d | G3P1L1A1 | COP | B+ | Neg | Neg | Neg | Neg | | |
| 27 | S | 29y | 1412565 | GNM | House Wife | 5y | 02.09.22 | 09.06.23 | 27w5d | Primi | IVF | O neg | Neg | Neg | Neg | Neg | | |
| 28 | SV | 27y | 1481253 | 12th | Dental Staff | 1y | 04.02.23 | 11.11.23 | 10w5d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 29 | MR | 33y | 696721 | 8th | House Wife | 5y | 22.01.23 | 29.10.23 | 17w5d | G4P1L1A2 | BOH | A neg | Neg | Neg | Neg | Neg | | |
| 30 | RB | 35y | 1336182 | 10th | House Wife | 6y | 06.02.23 | 13.11.23 | 10w4d | G3P1L1A1 | none | A+ | Neg | Neg | Neg | Neg | | |
| 31 | S | 37y | 1475579 | 8th | House Wife | 15y | 05.10.22 | 12.07.23 | 21w1d | G5P2IUD2L2 | BOH | B neg | Neg | Neg | Neg | Neg | | |
| 32 | MS | 29y | 717551 | MD | Anesthesiologist | 1y | 21.09.22 | 28.06.23 | 25w5d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 33 | M | 30y | 1160932 | Illiterate | House Wife | 6y | 30.06.22 | 06.04.23 | 37w4d | G2P1L1 | none | A+ | Neg | Neg | Neg | Neg | | |
| 34 | S | 35y | 1431411 | 12th | House Wife | 2y | 20.02.23 | 27.12.23 | 14w2d | G2P1L1 | none | B+ | Neg | Neg | Neg | Neg | | |
| 35 | GK | 29y | 1486880 | M.Tech | Engineer | 2y | 11.02.23 | 18.11.23 | 15w6d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 36 | SS | 32y | 276224 | B.Com | House Wife | 3y | 02.11.22 | 09.08.23 | 20w2d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 37 | A | 36y | 506549 | B. A. | House Wife | 5y | 30.12.22 | 06.10.23 | 12w5d | G4P2A1L2 | none | B+ | Neg | Neg | Neg | Neg | | |
| 38 | NK | 30y | 1490239 | 10th | House Wife | 10y | 19.12.22 | 25.09.23 | 15w4d | G5P2A2L2 | BOH | O neg | Neg | Neg | 3+ | Pos | 32 | anti-D |
| 39 | BK | 35y | 1489353 | 8th | House Wife | 12y | 13.12.22 | 19.09.23 | 17w2d | G3P2A1L1 | Hypothyroid | B neg | Neg | Neg | Neg | Neg | | |
| 40 | M | 38y | 1494945 | M. Com | House Wife | 16y | 16.08.22 | 23.05.23 | 34w3d | G4P2L1A1 | none | B neg | Neg | Neg | 4+ | Pos | 64 | anti-D |
| 41 | B | 32y | 1346242 | M.A. | House Wife | 1y | 01.12.22 | 08.09.23 | 19w6d | G2A1 | none | A+ | Neg | Neg | Neg | Neg | | |
| 42 | AP | 23y | 1431653 | Dietician | House Wife | 1y | 09.11.22 | 16.08.23 | 23w0d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 43 | J | 33y | 1501455 | 10th | House Wife | 9y | 10.08.22 | 17.05.23 | 36w0d | G2P1NND1 | Hypothyroid, uGDM | B neg | Neg | Neg | Neg | Neg | | |
| 44 | J | 34y | 168943 | 12th | House Wife | 6y | 24.11.22 | 31.08.23 | 20w6d | G2P1L1 | Hypothyroid | O neg | Neg | Neg | Neg | Neg | | |
| 45 | KK | 21y | 1484543 | Dip. Aviation | Air Hostess | 1y | 03.11.22 | 10.08.23 | 23w6d | Primi | Hypothyroid | O+ | Neg | Neg | Neg | Neg | | |
| 46 | SK | 30y | 1484755 | 12th | House Wife | 11y | 19.11.22 | 26.08.23 | 22w5d | G2A1 | none | O+ | Neg | Neg | Neg | Neg | | |
| 47 | ND | 32y | 937570 | 12th | House Wife | 3y | 09.01.23 | 16.10.23 | 15w3d | Primi | Hypothyroid, DM on insulin | AB neg | Neg | Neg | Neg | Neg | | |
| 48 | MK | 25y | 1506806 | Graduate | WFH job | 3y | 10.10.22 | 17.07.23 | 28w3d | G2P1L1 | Asthamatic | B+ | Neg | Neg | Neg | Neg | | |
| 49 | SS | 35y | 22419 | M.A. | House Wife | 9Y | 07.02.23 | 14.11.23 | 15w0d | G2P1L1 | none | O neg | Neg | Neg | 3+ | Pos | 256 | anti-D |

| S.NO. | Name | Age | MRD NO. | Edu. | Occ. | MF | LMP | EDD | GA | Obs Score | High Risk | ABO Group | DAT | AC | IAT | Ab. Screen | Ab. Titer | Ab. ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 50 | HK | 36y | 7894 | B.Sc. | House Wife | 5y | 31.05.22 | 07.03.23 | 21w4d | G2P1 | none | B neg | Neg | Neg | Neg | Neg | | |
| 51 | KK | 34y | 1142227 | M.A | House Wife | 5y | 25.05.22 | 01.03.23 | 35w0d | G3P1A1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 52 | P | 37y | 6546 | GNM | Staff Nurse | 8y | 19.08.22 | 26.05.23 | 27w4d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 53 | RK | 30y | 279907 | 10th | House Wife | 3y | 26.07.22 | 03.05.23 | 31w2d | G2P1L1 | none | A+ | Neg | Neg | Neg | Neg | | |
| 54 | RK | 26y | 1510177 | 12th | Private job | 1y | 02.10.22 | 10.07.23 | 30w2d | Primi | none | A+ | Neg | Neg | Neg | Neg | | |
| 55 | S | 32y | 9109 | 10th | House Wife | 4y | 08.12.22 | 15.09.23 | 20w5d | G2P1L1 | none | B+ | Neg | Neg | Neg | Neg | | |
| 56 | GK | 30y | 368387 | 12th | House Wife | 2y | 26.12.22 | 03.10.23 | 18w3d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 57 | MK | 27y | 515762 | B. Sc. | Staff Nurse | 3y | 03.01.23 | 10.10.23 | 14w2d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 58 | M | 30y | 1517148 | M. Tech | House Wife | 5y | 29.10.22 | 03.08.23 | 34w1d | G3P2A1 | none | B neg | Neg | Neg | 3+ | Pos | 256 | anti-D+C |
| 59 | PD | 28y | 1547301 | 12th | House Wife | 1y | 05.02.23 | 11.11.23 | 21w4d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 60 | U | 32y | 391300 | B.A. | Private job | 1y | 20.12.22 | 27.09.23 | 28w5d | Primi | none | O+ | Neg | Neg | Neg | Neg | | |
| 61 | LK | 36y | 8148 | Illiterate | House Wife | 7y | 07.12.22 | 14.09.23 | 30w1d | G3P2L2 | Hypothyroid | B+ | Neg | Neg | Neg | Neg | | |
| 62 | PK | 33y | 8376 | 10th | House Wife | 4y | 12.11.22 | 18.08.23 | 34w1d | G2P1L1 | none | B+ | Neg | Neg | Neg | Neg | | |
| 63 | SS | 30y | 1498825 | B Tech | IT job | 3y | 04.10.22 | 11.07.23 | 36w1d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 64 | NA | 27y | 1549046 | 12th | Private job | 2y | 29.12.22 | 06.10.23 | 27w3d | Primi | none | O neg | Neg | Neg | Neg | Neg | | |
| 65 | J | 34y | 762750 | 12th | House Wife | 6y | 20.01.23 | 27.10.23 | 24w5d | G2P1L1 | GDM on Oral meds | A+ | Neg | Neg | Neg | Neg | | |
| 66 | KK | 28y | 1494910 | 12th | House Wife | 3y | 31.11.22 | 07.09.23 | 33w6d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 67 | SK | 28y | 1568111 | 10th | House Wife | 3y | 08.01.23 | 15.10.23 | 30w3d | Primi | none | A neg | Neg | Neg | Neg | Neg | | |
| 68 | NW | 28y | 1568343 | M.A. | House Wife | 5y | 18.01.23 | 25.10.23 | 29w4d | G2A1 | none | B neg | Neg | Neg | Neg | Neg | | |
| 69 | KK | 37y | 179934 | 10th | House Wife | 13y | 19.02.23 | 24.11.23 | 25w3d | G2P1L1 | none | B neg | Neg | Neg | Neg | Neg | | |
| 70 | S | 29y | 1569801 | 12th | Private job | 3y | 22.12.22 | 29.09.23 | 33w1d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 71 | GA | 28y | 1516659 | ANM | House Wife | 2y | 28.06.23 | 05.04.24 | 10w2d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 72 | RKS | 31y | 294052 | 10th | House Wife | 7y | 08.03.23 | 15.12.23 | 22w3d | G3P2L2 | none | O+ | Neg | Neg | Neg | Neg | | |
| 73 | MS | 29y | 1538356 | M.A | Student | 1y | 30.01.23 | 07.12.23 | 23w4d | Primi | none | A+ | Neg | Neg | Neg | Neg | | |
| 74 | A | 30y | 539691 | B.BA | House Wife | 2y | 12.01.23 | 20.10.23 | 30w5d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 75 | MD | 27y | 1575339 | B.A. | House Wife | 3y | 24.02.23 | 03.12.23 | 25w2d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 76 | SK | 21y | 1580350 | 8th | House Wife | 1y | 28.04.22 | 02.02.23 | 17w5d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 77 | S | 28y | 1458806 | B.A. | House Wife | 3y | 07.04.22 | 13.01.23 | 20w3d | G2P1L1 | none | A neg | Neg | Neg | Neg | Neg | | |
| 78 | RK | 32y | 1580485 | 12th | House Wife | 6y | 28.05.22 | 04.03.23 | 13w2d | G2P1L1 | COP | B+ | Neg | Neg | Neg | Neg | | |
| 79 | RK | 29y | 1582805 | 12th | Private job | 5y | 24.05.23 | 01.03.24 | 14w3d | G3P2L2 | none | O+ | Neg | Neg | Neg | Neg | | |
| 80 | SR | 25y | 1585115 | B.A. | Teacher | 4y | 31.03.23 | 06.01.24 | 22w5d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 81 | CK | 34y | 1585004 | M.A | House Wife | 5y | 18.02.23 | 25.11.23 | 28w5d | G2P1L1 | none | O+ | Neg | Neg | Neg | Neg | | |
| 82 | PC | 34y | 38796 | Lab Tech | Private job | 5y | 08.02.23 | 17.10.23 | 34w2d | G2P1L1 | GDM on Oral meds | B neg | Neg | Neg | Neg | Neg | | |
| 83 | A | 29y | 1537177 | 12th | House Wife | 3y | 27.01.23 | 05.12.23 | 28w1d | G2A1 | none | O+ | Neg | Neg | Neg | Neg | | |
| 84 | P | 28y | 1591549 | M.Sc | Student | 2y | 31.01.23 | 08.11.23 | 32w5d | Primi | none | AB+ | Neg | Neg | Neg | Neg | | |
| 85 | HK | 32y | 202611 | 10th | House Wife | 5y | 29.05.23 | 06.03.24 | 16w2d | G2A1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 86 | MK | 28y | 1586067 | 12th | House Wife | 2y | 01.04.23 | 08.01.24 | 22w4d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 87 | KKD | 24y | 1123046 | 12th | Private job | 1y | 10.06.23 | 18.03.24 | 13w3d | Primi | none | A+ | Neg | Neg | Neg | Neg | | |
| 88 | T | 28y | 738752 | B.Sc. | Staff Nurse | 4y | 28.03.23 | 04.01.24 | 25w6d | G2P1L1 | none | A neg | Neg | Neg | Neg | Neg | | |
| 89 | AI | 25y | 932189 | B. A. | Student | 3y | 19.06.23 | 26.03.24 | 14w4d | Primi | none | B+ | Neg | Neg | Neg | Neg | | |
| 90 | AJ | 32y | 1601836 | B.Tech. | Govt. Job | 5y | 07.05.23 | 12.02.24 | 21w2d | G2A1 | none | A neg | Neg | Neg | Neg | Neg | | |
| 91 | AK | 35y | 1602850 | 10th | House Wife | 6y | 08.04.23 | 16.01.24 | 25w4d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 92 | JK | 36y | 1591392 | 8th | House Wife | 15y | 07.03.23 | 14.12.23 | 31w1d | G4P2A1L2 | none | AB neg | Neg | Neg | Neg | Neg | | |
| 93 | HV | 27y | 1608783 | Illiterate | House Wife | 7y | 29.06.23 | 07.04.24 | 15w2d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 94 | N | 29y | 1590010 | 12th | House Wife | 6y | 07.04.23 | 13.01.24 | 28w1d | G3P2L2 | none | O neg | Neg | Neg | Neg | Neg | | |
| 95 | MK | 38y | 1615055 | 8th | House Wife | 16y | 05.04.23 | 11.01.24 | 29w2d | G4P1A2L1 | GDM on Oral meds, BOH | O neg | Neg | Neg | Neg | Neg | | |
| 96 | AA | 28y | 1622472 | 12th | House Wife | 1y | 08.05.23 | 14.02.24 | 28w4d | Primi | none | O neg | Neg | Neg | Neg | Neg | | |
| 97 | SK | 32y | 1310008 | B.A. | Teacher | 4y | 07.08.23 | 15.05.24 | 16w5d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 98 | TJ | 26y | 1362281 | 12th | Private job | 2y | 31.05.23 | 09.03.24 | 26w3d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |

| S.NO. | Name | Age | MRD NO. | Edu. | Occ. | MF | LMP | EDD | GA | Obs Score | High Risk | ABO Group | DAT | AC | IAT | Ab. Screen | Ab. Titer | Ab. ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99 | RK | 37y | 1634620 | 12th | House Wife | 9y | 19.05.23 | 26.02.24 | 28w2d | G3P1A1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 100 | GK | 33y | 1634663 | 10th | House Wife | 3y | 28.04.23 | 04.02.24 | 31w3d | G2A1 | none | B neg | Neg | Neg | Neg | Neg | | |
| 101 | LK | 31y | 1639615 | 12th | Private job | 2y | 14.08.23 | 22.05.24 | 17w3d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 102 | HK | 38y | 1641329 | 8th | House Wife | 11y | 15.04.23 | 21.01.24 | 35w2d | G2P1L1 | none | B neg | Neg | Neg | Neg | Neg | | |
| 103 | MK | 34y | 1315969 | 10th | House Wife | 4y | 25.05.23 | 04.03.24 | 29w5d | G2A1 | Hypothyroid | B neg | Neg | Neg | Neg | Neg | | |
| 104 | S | 29y | 966985 | B.Com | House Wife | 1y | 12.07.23 | 20.04.24 | 23w1d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 105 | RK | 26y | 1538347 | B.A. | Fashion design | 5y | 31.05.23 | 09.03.24 | 30w6d | G2P1L1 | none | A neg | Neg | Neg | 4+ | Pos | 512 | anti-D |
| 106 | SK | 31y | 1649688 | 12th | House Wife | 3y | 13.06.23 | 21.03.24 | 29w3d | G2A1 | none | B neg | Neg | Neg | 3+ | Pos | 256 | anti-D |
| 107 | GK | 41y | 1648606 | M.Sc | Staff Nurse | 8y | 14.09.23 | 22.06.24 | 16w1d | G2L1 | GDM on Oral meds | O+ | Neg | Neg | Neg | Neg | | |
| 108 | KK | 28y | 1645278 | 10th | House Wife | 6y | 25.09.23 | 03.07.24 | 14w5d | G3P2L2 | none | B+ | Neg | Neg | Neg | Neg | | |
| 109 | AK | 30y | 1652411 | B.A. | House Wife | 4y | 27.09.23 | 05.07.24 | 15w0d | G2P1L1 | none | O+ | Neg | Neg | Neg | Neg | | |
| 110 | NS | 32y | 1651526 | C.A. | Bank | 2y | 27.05.23 | 07.03.24 | 32w1d | Primi | COP | B neg | Neg | Neg | Neg | Neg | | |
| 111 | JA | 28y | 1652860 | 12th | Student | 3y | 04.07.23 | 12.04.24 | 27w1d | Primi | none | A neg | Neg | Neg | Neg | Neg | | |
| 112 | BD | 43y | 1650873 | Illiterate | House Wife | 18y | 20.09.23 | 29.06.24 | 16w4d | G5P3A1L3 | GDM uncontrolled | O+ | 1+ | Neg | Neg | Neg | | |
| 113 | KKB | 43y | 1649430 | 12th | House Wife | 15y | 05.09.23 | 15.06.24 | 19w3d | G3P2L2 | none | O neg | Neg | Neg | Neg | Neg | | |
| 114 | P | 26y | 1345887 | 12th | House Wife | 3y | 21.07.23 | 01.05.24 | 26w1d | Primi | none | A neg | Neg | Neg | Neg | Neg | | |
| 115 | SR | 29y | 1662514 | M.Sc | Student | 4y | 25.10.23 | 05.08.24 | 14w0d | G2P1L1 | none | O neg | Neg | Neg | Neg | Neg | | |
| 116 | HS | 32y | 1664216 | 10th | House Wife | 5y | 12.06.23 | 23.03.24 | 33w5d | G3P2L2 | Hypothyroid | A neg | Neg | Neg | Neg | Neg | | |
| 117 | JK | 28y | 1670156 | M.Com | Student | 5y | 26.10.23 | 06.08.24 | 14w6d | G2P1L1 | none | O+ | Neg | Neg | Neg | Neg | | |
| 118 | M | 27y | 1674360 | 10th | House Wife | 1y | 14.11.23 | 24.08.24 | 13w5d | Primi | none | B neg | Neg | Neg | Neg | Neg | | |
| 119 | A | 27y | 1665205 | 12th | House Wife | 3y | 22.08.23 | 01.06.24 | 25w0d | G2P1L1 | none | A neg | Neg | Neg | Neg | Neg | | |

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**No. BFUHS/2K23p-TH/** 3935 **Date** 14/03/2024

DAYANAND MEDICAL COLLEGE & HOSPITAL * LUDHIANA *
Receipt No. 3519
Date 18/3/24
Principal's Office

**The Principal**
**Dayanand Medical College & Hospital**
**Ludhiana**

**Subject:: Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of **Jannet Mary John**, a student of your college for **MD Immuno Haematology & Blood Transfusion** along with the names of Supervisor/Co-Supervisor as under:-

**Topic Plan of Thesis**
Prevalence of clinically significant red cell alloantibodies in pregnant women.

**Supervisor**
Dr. Rajesh Kumar
Professor & Head
Dept. Of I.H.B.T

**Co-Supervisor:-**
Dr. Pooja Tandon
Assoc. Prof.
Dept.of Obstetrics & Gynaecology

The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

*Note :*

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

All Concerned
18/3/2024

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(31)

**No. BFUHS/2K23p-TH/** 13221 **Date** 29/12/23

**The Principal**
**Dayanand Medical College & Hospital**
**Ludhiana**

**Subject:: Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of **Jannet Mary John**, a student of your college for **MD Immuno Haematology & Blood Transfusion** along with the names of Supervisor/Co-Supervisor as under:-

**Topic Plan of Thesis**
Prevalence of clinically significant red cell alloantibodies in pregnant women .

**Supervisor**
Dr. Amarjit Kaur
Professor & Head.
Dept. of I.H.B.T.

**Co-Supervisor:-**
Dr. Pooja Tandon
Assoc. Prof.
Dept.of Obstetrics Co & Gynaecology

Co-Supervisor
Dr. Rajesh Kumar
Professor
Dept. Of I.H.B.T

The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

*Note :*

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

# PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN

**THESIS PROPOSAL**

**FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS**

**FOR THE DEGREE OF**

**Doctor of Medicine**

**In**

**IMMUNO HEMATOLOGY & BLOOD TRANSFUSION**

**OF THE**

**BABA FARID UNIVERSITY OF HEALTH SCIENCES**
**FARIDKOT**

**JUNE 2022**

**DR. JANNET MARY JOHN**

**DEPARTMENT OF IMMUNO HEMATOLOGY & BLOOD TRANSFUSION**
**DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA**
**LUDHIANA, PUNJAB-141001**

# ABSTRACT OF PLAN OF THESIS

| | |
|---|---|
| Title | PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN |
| For the degree of | MD IMMUNO HEMATOLOGY & BLOOD TRANSFUSION |
| Name of the candidate | DR. JANNET MARY JOHN |
| Supervisor | DR. AMARJIT KAUR, PROFESSOR & HEAD<br>DEPARTMENT OF IMMUNO HEMATOLOGY & BLOOD TRANSFUSION |
| Co-supervisors | DR. RAJESH KUMAR, PROFESSOR<br>DEPARTMENT OF IMMUNO HEMATOLOGY & BLOOD TRANSFUSION<br>DR. POOJA TANDON, ASSOCIATE PROFESSOR<br>DEPARTMENT OF OBSTETRICS AND GYNECOLOGY |
| Institution | DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA |
| University | BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT |

**Introduction:** Hemolytic disease of fetus and newborn (HDFN) is defined as anemia and hyperbilirubinemia caused by an incompatibility between maternal and fetal red blood cells (RBCs). In 98% cases it is caused due to ABO and Rh incompatibility and antibodies to other blood group antigens (Kell, c, E, C, Kidd, Duffy and M) are causative in remaining 2%. Red cell antibody screening (RCAS) is a valuable tool in the detection of alloantibodies to other blood group systems (other than ABO and Rh) in the serum of patients during pregnancy.

**Aim of Study:** To study the prevalence of clinically significant red cell alloantibodies in pregnant females.

**Materials & methods:** All antenatal women registered will be typed for ABO-Rh antigens. Screening and identification for irregular antibodies will be done using a 3-cell and 11-cell panel respectively and records will be reviewed.

**Clinical Significance:** To reiterate the importance of maternal red cell antibody screening during early pregnancy to help identify and manage high-risk pregnancies.

**Keywords:** antibodies, HDFN, alloimmunization, screening

(Signature)
Candidate

(Signature)
Co-supervisor

(Signature)
Co-supervisor

(Signature)
Supervisor

(Signature)
Principal

## CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN"** exist in the Department of Immuno Hematology & Blood Transfusion at DMC&H, Ludhiana and will be provided to be candidate of will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate herself under our supervision and guidance.

Supervisor

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Professor & Head
Department of Immuno Hematology
& Blood Transfusion
DMCH, Ludhiana

Co-Supervisors

**Dr. RAJESH KUMAR**
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**Head of the Department**

**Dr. AMARJIT KAUR**
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Place: DMCH, LUDHIANA
Date: 30/09/22

## APPROVAL PROFORMA
## BY RESEARCH & ETHICAL COMMITTEE
## DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

| | |
|---|---|
| **Name of candidate** | DR. JANNET MARY JOHN |
| **Department** | IMMUNO HEMATOLOGY & BLOOD TRANSFUSION |
| **Topic of Thesis** | PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN |
| **Likely date of appearing in PG examination** | APRIL - MAY 2025 |
| **Date of enrollment** | 30TH APRIL 2022 |
| **Name of Head of Department** | DR. AMARJIT KAUR |
| **1. Supervisor**<br>**2. Co-Supervisors** | DR. AMARJIT KAUR<br>DR. RAJESH KUMAR<br>DR. POOJA TANDON |
| **Signature of Members of Research Committee with Stamp** | **Signature of Members of Ethics Committee with Stamp** |
| Dr. Harpreet Kaur<br>(Professor & Head, Pathology) | Mr. R K Sharma<br>(Legal Expert) |
| Dr. Jaspal Singh<br>(Professor & Head, Surgery) | Dr. Ramit Mahajan<br>(Associate Professor, Gastroenterology) |
| Dr. Dinesh Gupta<br>(Professor & Head, Medicine) | Dr. Anu Sharma<br>(Professor, Anatomy) |
| Dr. Sandeep Kaushal<br>(Dean Academics & Professor & Head, Pharmacology) | Dr. Sandeep Kaushal<br>(Dean Academics & Professor & Head, Pharmacology) |
| Dr. Gurpreet S Wander<br>(Vice Principal) | Dr. Gurpreet S Wander<br>(Vice Principal & Professor & Head, Cardiology) |
| | Dr. Gagandeep Singh<br>(Secretary, Ethics Committee Professor & Head, Neurology) |

**Approved: Yes / ~~No~~**

**Dr. Sandeep Puri**
**Chairperson**
**Research Committee**

Chairperson
Research Committee

**Approved: Yes / ~~No~~**

**Prof. Arvind Malhotra**
**Chairperson**
**Ethics Committee**

Chairperson
Ethics Committee

# BABA FARID UNIVERSITY OF HEALTH SCIENCES
# FARIDKOT
# PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

| | | |
|---|---|---|
| 1 | NAME OF THE CANDIDATE AND ADDRESS | DR. JANNET MARY JOHN<br>MCB - Z - 5 - 10991,<br>NEAR INDERPRASTH GATE,<br>VISHWAS NAGAR, OPP. LAKE NO. 3,<br>P.O. NFL TOWNSHIP, GONIANA ROAD, BATHINDA, PUNJAB-151003 |
| 2 | NAME OF THE INSTITUTE | DAYANAND MEDICAL COLLEGE AND HOSPITAL, LUDHIANA |
| 3 | COURSE OF STUDY AND SUBJECT | MD IMMUNO HEMATOLOGY & BLOOD TRANSFUSION |
| 4 | DATE OF ADMISSION OF COURSE | 30/ 04/ 2022 |
| 5 | TITLE OF THE TOPIC | PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN |

## 6. BRIEF RESUME OF THE INTENDED WORK

### 6.1 NEED FOR THE STUDY

Red cell immunization during pregnancy is an obstacle that continues to challenge obstetricians and blood transfusionists even after 60 years of introduction of Rhesus (Rh) D prophylaxis. Before the introduction of RhIG, maternal anti-D alloimmunization was the most common cause of Hemolytic Disease of the Fetus and Newborn (HDFN). With the arrival of anti-D prophylaxis, there were hopes that the numbers of HDFN due to D antigen incompatibility will come down. Several possible pathways of the mechanism of action of RhIG have been hypothesized and thoroughly studied in the past decades; however the exact mechanism remains unknown.[1]

Nevertheless, having reached the 21st century, the burden of alloimmunization in pregnancy is still a problem that needs to be tackled. Blood group antigens of Rh system other than D antigen (C, c, E, e, $C^w$) and other blood group systems have come into spotlight. The alloimmunization in pregnancy has been studied extensively in different parts of the world. The antenatal immunohematological work up of pregnant women requires the investigation of unexpected RBC antibodies in their sera during the pregnancy. When RBC antibody screening is positive, it is necessary to determine specificity of the antibody, its clinical importance and the ability to cross the placenta to cause HDFN.[2]

Hemolytic Disease of the Fetus and Newborn (HDFN) is a serious complication in pregnancy which occurs in case of antigenic difference between maternal and fetal RBCs. The ability of individuals to produce antibodies in response to antigenic exposure varies, depending on complex genetic factors that are not entirely defined. Clinically significant red cell alloantibodies are those that have the potential to cause hemolysis of the red blood cells bearing the corresponding antigen. Maternal antibodies capable of causing HDFN could either be ABO antibodies or non-ABO alloantibodies that can develop in the mother due to

sensitization following a blood transfusion or previous pregnancy with red cell antigenic differences.[3]

Immunoglobulin class and subclass of the maternal antibody affects the severity of the HDFN. Of the immunoglobulin classes (i.e., IgG, IgM, IgA, IgE, and IgD), only IgG is transported across the placenta. The active transport of IgG begins in the second trimester and continues until birth. These maternal antibodies cross the placenta to induce hemolysis resulting in fetal or neonatal anemia, hyperbilirubinemia and sometimes even fatal hydrops fetalis. It may also cause death if left untreated. Hemolysis occurs when maternal IgG attaches to specific antigens of the fetal RBCs. The antibody-coated cells are removed from the circulation by the macrophages of the fetal spleen. The rate of RBC destruction depends on antibody titer and the number of antigenic sites on the fetal RBCs.

HDFN is most frequently caused by antibodies with anti-D specificity, followed by anti-K, anti-c, anti-E, other Rh antibodies, or exceptionally anti-Fy (Duffy) or anti-Jk (Kidd). Thus far no immunoprophylaxis has been developed to prevent non-D alloimmunization, however the clinical relevance of implementing anti-KEL anti-HPA-1a immunoglobulins have been investigated in murine models.

As per the guidelines of the British Committee for Standards in Hematology, all the pregnant women should be ABO and D antigen typed and screened for the presence of red cell antibodies early in pregnancy and at the 28th week of gestation. The guidelines in Netherlands make it mandatory to screen all pregnant women for the presence of irregular antibodies in the first trimester of pregnancy. Thus, in most of the developed countries, universal screening for irregular erythrocyte antibodies, of all the antenatal females, including D antigen-positive pregnant ones is compulsory. However, there are no standardized guidelines being followed in the developing countries. Moreover, the published data shows a wide range of variation in the rates of alloimmunization among different geographic areas. It is universally

considered that there should be evidence based guidelines for screening alloantibodies in pregnant women in developing countries such as India for the proper management of child birth.[4] Pregnant women with known RBC alloimmunization and/or a history of HDFN should be closely monitored using antibody titers. The fetus also needs a close monitoring throughout the pregnancy to check for fetal well-being and fetal anemia; and to determine the best time for delivery.

## 6.2 REVIEW OF LITERATURE

The ABO blood grouping system was discovered by Landsteiner and his coworkers von Castello and Sturli. With the introduction of blood grouping tests by Ottenberg, the fatal rates of blood transfusions drastically fell. Unger described the possibility of agglutination reaction between the donor cells and the patient's serum, due to the existence of irregular antibodies. Alloantibody identification which was introduced by Coombs et al, added a new level of safety to blood transfusions. All these discoveries led to the identification of the 346 blood group antigens, which are categorized into 35 blood group systems and 38 low- and high-frequency antigens, as they do not fulfill the criteria for the blood group classification.

With the entry of an incompatible antigen into an immunocompetant host, an immune response is triggered. This leads to alloimmunization. Immune responses can be categorized as follows:

a. Thymus independent: consists mostly of carbohydrate antigens that produce IgM antibodies occurring naturally or derived from the environment. Interaction with these, lead to intravascular type of hemolysis.

b. Thymus dependent: consists mostly of protein antigens that produce IgG antibodies. Interaction with these, lead to extravascular hemolysis.

Among the various blood group systems discussed, the most important antibodies that lead to alloimmunization are Rh (anti-D, -C, -E, -c and -e), KEL (anti-K), FY (anti-Fya and anti-Fyb), JK (anti-Jka and anti-Jkb) and the MNS (anti-M, -S and -s). Out of these, the anti-D is considered to be the most immunogenic. After transfusion of D-positive erythrocytes, around 80% of immunocompetant D-negative people become alloimmunized. Thus, in order to prevent this, testing of anti-D before transfusion is mandatory.[5]

Anti-D occurring in Rh negative women was a major cause for severe HDFN worldwide until the 1960s. Following successful implementation of prophylaxis, changes in birth order and improved quality of medical care, morbidity and mortality due to Rh(D) related HDFN in many countries drastically reduced from 12%-13% to 1%-2%[1]. Meanwhile, other irregular antibodies that were found to cause HDFN, principally anti-c, anti-E, and antibodies to antigens of Kell, Kidd, Duffy and MNS blood group systems, gained prominence.[6]

Currently, the availability of wider screening panels has enabled the detection of various minor blood group antibodies, some of which are well known to have relevance in the antenatal setting. In developing countries, like India the financial burden of routine screening for irregular antibodies, has to be weighed against the benefits. Thus routine screening of these irregular antibodies may not be possible. Antibodies to s, p, Kidd, and Duffy are known to cause mild to severe degree of HDFN whereas those to S, I, k, Lutheran, and Kell are known to cause mild to moderate degree of hemolysis.

Maternal sensitization or isoimmunization occurs in response to foreign erythrocyte surface antigens leading to the formation of IgG type of antibodies. The most common routes of alloimmunization are through blood transfusions, fetomaternal hemorrhage due to delivery, other interventional procedure, ectopic pregnancy, or abortion. During pregnancy, antibodies produced due to alloimmunization cross placenta and cause hemolysis of fetal red cells and thereby leading to anemia. More severe stage due to deranged fetal metabolism leads to hydrops fetalis. With anti-D immunoprophylaxis, the frequency of alloimmunization of the mother has severely reduced resulting in better fetal outcome and prognosis. Moreover, due to recent advancement in fetal surveillance, outcomes of such conditions have improved to a great extent.[5]

DIAGNOSIS OF HDFN CAUSED BY RBC ALLOIMMUNIZATION

ABO, RhD, and Antibody Screen

The prenatal specimen must be typed for ABO and RhD. The antibody detection method, or indirect antihuman globulin test (IAT), must be able to detect clinically significant IgG alloantibodies that are reactive at 37°C and in the anti - globulin phase. At least two separate reagent screening RBCs that express all of the common blood group antigens (preferably homozygous) should be used. For tube testing, an antibody-enhancing medium such as polyethylene glycol (PEG) or low ionic strength solution (LISS) can increase sensitivity of the assay. Prenatal patients may produce clinically insignificant anti bodies, such as anti-Lea or anti Leb. Therefore, immediate spin and room temperature incubation phases can be omitted, and anti-IgG rather than polyspecific antiglobulin reagent is used. These steps reduce detection of IgM antibodies, which cannot cross the placenta. Other antibody screening methods, such as solid phase or gel column, may be used. If the antibody screen is nonreactive, repeat testing is recommended before RhIG therapy in RhD-negative prenatal patients and in the third trimester if the patient has been transfused or has a history of unexpected antibodies.[7]

Antibody Identification

If the antibody screen is reactive, the antibody identity must be determined. Follow-up testing will depend on the antibody specificity. Cold reactive IgM antibodies such as anti-I, anti-IH, anti-Lea, anti-Leb, and anti-P1 can be ignored. Lewis system antibodies are rather common in pregnant women but have not been reported to cause HDFN. Antibodies such as anti-M and anti-N can be IgM or IgG or a combination of both. Both anti-M and anti-N can cause mild to moderate HDFN, although rarely. To establish the immunoglobulin class, the serum can be treated with a sulfhydryl reagent, such as dithiothreitol or 2-mercaptoethanol, and then retested with appropriate controls. The J-chain of IgM anti- bodies will be destroyed by this treatment; IgG antibodies will remain reactive. Many Rh-negative pregnant women have weakly reactive anti-D, particularly during the third trimester. Most of these women have received RhIG,

either after an event with increased risk of fetomaternal hemorrhage or at 28 weeks' gestation (antenatal). The passively administered anti-D will be weakly reactive in testing and will remain demonstrable for 2 months or longer. This must be distinguished from active immunization. A titer higher than 4 almost always indicates active immunization; with a titer under 4, active immunization cannot be ruled out, but it is less likely. If encountering a sample from a pregnant patient, using PEG appears to provide the least number of false positives compared to gel cards and solid phase.[8] Communication with the patient's provider to obtain a history of RhIG administration is an important confirmatory step as serological methods alone are not able to determine the derivation of the antibody. If the antibody specificity is determined to be clinically significant and the antibody is IgG, further testing is required. Other than anti-D, the most common and most significant antibodies are anti-K, anti-E, anti-c, anti-C, and anti-Fya.

Antibody Titers

The relative concentration of all antibodies capable of crossing the placenta and causing HDFN is determined by anti-body titration. The patient serum or plasma is serially diluted and tested against appropriate RBCs to determine the highest dilution at which a reaction occurs.[9] The method must include the indirect antiglobulin phase using anti-IgG reagent. The result is expressed as either the reciprocal of the titration endpoint or as a titer score. The titration must be performed exactly the same way each time the patient's serum is tested. The recommended method is the saline antiglobulin tube test, with 60-minute incubation at 37°C and the use of anti-IgG reagent, although other methods have also been proposed. The RBCs used for each titration should have the same genotype (preferably homozygous for the antigen of interest), approximately the same storage time, and the same concentration. The first serum or plasma specimen should be frozen and run in parallel with later specimens to increase accuracy since the titer test results are difficult

to reproduce.[10] Only a difference of greater than 2 dilutions or a score change of more than 10 is considered a significant change in titer. The method chosen is critical for the appropriate clinical correlation. Methods using enhancing media or gel columns result in higher titers, as shown by comparative proficiency testing. Therefore, the critical titer level for these other methods must be determined by the individual laboratory by reviewing the outcome of several pregnancies complicated by HDFN, if this method is to be used. For the recommended method, 16 is considered the critical titer. If the initial titer is 16 or higher, a second titer should be done at about 18 to 20 weeks' gestation. A titer reproducibly and repeatedly at ≥32 is an indication for color Doppler imaging to assess middle cerebral artery peak systolic velocity (MCA-PSV) after 16 weeks' gestation. When the titer is less than 32, it should be repeated at 4-week intervals, beginning at 16 to 20 weeks' gestation and then every 2 to 4 weeks during the third trimester. Antibody titer alone cannot predict severity of HDFN. In some sensitized women, the antibody titer can remain moderately high throughout pregnancy while the fetus is becoming more severely affected. Similarly, a previously sensitized woman can have consistently high antibody titer, whether pregnant or not, and whether the fetus is RhD-positive or RhD-negative. In others, the titer can rise rapidly, which portends increasing severity of HDFN. However, antibody titers consistently below the laboratory's critical titer throughout the pregnancy reliably predict an unaffected or mild-to-moderately affected fetus, with the exception of anti-K with a K-positive fetus. Titration studies at time of delivery are not recommended because they provide no clinically useful information.

De Vrijer et al[11] put forward that a total of 2392 pregnant women were screened for irregular erythrocyte antibodies (IEA) during a 2.5-year period (August 1995-January 1998): 2204 multiparous women and 188 primigravidae women. In 65 women 81 IEA were discovered. In the group

of 30 children positive for the antigen involved, 12 (40%) had clinical symptoms of HDN; intrauterine death was diagnosed once, one child died immediately after delivery. One child had signs of hydrops fetalis and two children needed an exchange transfusion. Most cases of HDN were caused by anti-D, anti-Kell and anti-c antibodies. First-trimester screening for IEA is recommended as it can be of help in early diagnosis and treatment of HDN.

As per Hoppe B et al,[12] a total of 22,782 pregnant and 12,903 non-pregnant women were analyzed. The antibody screen test was positive in 547 (2.40%) of the pregnant women. The positive test results were related to passive anti-D in 341 cases (1.50%), to clinically relevant alloantibodies in 43 cases (0.19%), to clinically irrelevant antibodies in 138 cases (0.61%), to autoantibodies in 23 cases (0.1%), and to autoantibodies plus alloantibodies in 2 cases (0.01%).

According to Lee et al,[13] of 28303 (21327 Chinese) antenatal attendances from 1997 to 2001, 213 (0•79%) women were found to have a total of 230 irregular antibodies. The study showed that the specificities of the antibodies differ with the commonest antibodies encountered; these being anti-Mi (57•6%), anti-E (19•7%), anti-S (10•6%) and anti-c (7•6%). The findings support the previous recommendation that routine antenatal antibody screening for Chinese women may not be worthwhile except in Rh(D)-negative subjects or those with an antecedent history of hemolytic disease of the newborn (HDN).

As per Shanthala AM et al[14] HDFN is caused due to ABO and Rh incompatibility in 98% cases and antibodies to other blood group antigens (Kell, c, E, C, Kidd, Duffy, M, and so on) are causative in remaining 2%. The most common antibody identified remained anti-D. In 2 cases of Rh negative pregnancy, the red cell antibody screening (RCAS) was suggestive of anti-D. Red cell antibody identification (RCAI) done, showed a combination of anti-D and anti-C.

Koelewijin JM et al[15] put forward that first trimester screening enables timely treatment of HDFN caused by antibodies other than anti-D. Overall, severe HDFN, requiring intrauterine or postnatal (exchange) transfusions, occurred in 3.7% of fetuses at risk: for anti-K in 11.6%; anti-c in 8.5%; anti-E in 1.1%; Rh antibodies other than anti-c, anti-D, or anti-E in 3.8%; and for antibodies other than Rh antibodies or anti-K, in none of the fetuses at risk. Five of 8 missed cases were caused by anti-c, with delay-induced permanent damage in at least 1.

Al-Ibrahim et al[16] stated that the largest fraction of alloimmunization involved Rh antigens (52.38%), while other groups such as Kell & Duffy play a less common role. Five types of alloantibodies were identified in addition to nonspecific-autoantibodies. The most frequent (52.38%) were against Rhesus 2.38%; Kell 2.38%; Duffy 2.38%; 4.76% were non-specific antibodies and 33.3% were autoantibodies. Alloimmunization are: anti-D 28.57%, anti-C 4.76% anti-E 14.28% and anti-e 4.76%; only one 2.38% developed anti-K; anti-Jk, one 2.38%; one had anti-Le 2.38%; there was one 2.38% with anti-Fy.

According to Pahuja S et al,[4] the overall prevalence of alloantibodies was 1.25% in the study population. There was a statistically significant difference between alloimmunisation rates in the D antigen-negative and D antigen-positive groups (10.7% versus 0.12%, respectively). Anti-D antibody contributed to 78.4% of total alloimmunisations.

As per Varghese J et al,[6] screening was performed on 5347 women, 339 (6.34%) of whom were Rh negative. Allosensitization was found in 79 women (1.48%; confidence interval 1.17 -1.84). In 29 of these 79 (37%) women the alloantibodies could not be identified. In the remaining 50 women, 54 antibodies were characterized. A total of 40 clinically significant antibody specificities were identified among 36 women, of whom four were Rh(D) positive. Anti D was the most frequent antibody found in 8.85 per cent Rh(D) negative women. The remaining

clinically significant antibodies identified included anti-C, c, E, Jka, Jkb, M and S.

Suresh B et al[17] stated that the overall prevalence of alloantibodies was 1.1%. There was a statistically significant difference between alloimmunization rates in the Rh D-antigen negative and D-antigen positive women (12.8% versus 0.3%). The antibodies detected in this study were, anti-D (63.8%), anti-D+C (13.7%), anti-C, anti-E, anti-M, anti-Lea, and anti-Leb (4.5% each). Anti-D contributed to 77.3% of total alloimmunization in this study.

As per Zwiers C et al,[1] a total of 11.9% of the 232 including immunized women had a possible ABO incompatibility in their first pregnancy (vs. expected 19.4%; 95% confidence interval [CI], 7.3-18.8; p 5 0.036). Furthermore, 1.0% women with non-Rh antibodies were D–, delivered a D1 child, and had therefore received RhIG, whereas 9.9% was expected (95% CI, 0.18-5.50; p 5 0.003).

Moinuddin I et al[3] said that, out of the 4545 included females, 440 had a positive red cell antibody screen. Of these 440 females, 34 had clinically significant alloantibodies, giving an overall prevalence of 0.74%. Anti-E was the most frequently identified significant alloantibody followed by anti-K. The most prevalent significant alloantibodies in RhD positive and RhD negative females were anti-E and anti-K, respectively.

According to Rahimi-Levene N et al,[18] 90948 women representing 70% of all births during 2011 were analyzed. Antibody screen was positive in 5245 (5.8%) women. Alloantibodies, excluding anti-D titer (<16) were identified in 900 (1.0%) women. Of 191 D– women, 75 (39.3%) had anti-D titer of 16 or greater. Other common clinically significant antibodies were anti-E (204, 23%), anti-K (145, 16%), and anti-c (97, 10.8%) alone or in antibody combinations. Multiple alloantibodies were observed in 132 of 900 (15%) of women. Severe HDFN developed in 6.8% (9/132) of these pregnancies.

Li S et al[19] stated that anti-D was the most common cause of HDF, followed by anti-M. No anti-K- or isolated anti-c-associated HDF was found. The incidence of severe HDF was higher in the group with anti-D combined with other antibodies than in the group with anti-D alone (P=0.025), but no significant difference was found in hemoglobin level and reticulocyte count in the anemic fetuses between these two groups. A high maternal antibody titre (P<0.001), multiple affected pregnancies (P<0.001) and other single-antibody (P=0.042) were independent risk factors for HDF. A higher reticulocyte count (P=0.041) was an independent risk factor for severe HDF in anemia fetuses affected by Rh(D) alloimmunization.

Dholakiya SK et al[5] put forward that out of total 8920 multigravida females, 8488 were D‑antigen positive whereas 432 were D‑antigen negative. A total of 126 antibodies among 117 females (1.31%) were found; out of them, 33 were found in D‑antigen positive females (0.39%) and 84 in D‑antigen negative ones (19.44%) looking at overall frequency of other antibodies such as anti‑C: 9, anti‑c: 9, anti‑E: 13, anti‑Cw: 1, anti‑M: 5, anti‑S: 8, anti‑Fya: 3, and anti‑D: 78; it was found that anti‑D is the most common.

## 6.3 AIMS AND OBJECTIVES OF THE STUDY

- To study the prevalence of clinically significant red cell alloantibodies in pregnant females.

## 7. MATERIALS AND METHODS

The study includes all antenatal patients, irrespective of their period of gestation and obstetric history. Ethylenediamine tetraacetic acid (EDTA) sample will be used for ABO-Rh grouping and Gel vial sample for screening antibodies using polyspecific antihuman globulin gel cards (anti IgG+C3d) and a 3 cell and 11 cell panel (Diapanel, Bio-Rad, Switzerland). Once the antibody is identified, titration will be carried out. Direct Agglutination Test (DAT) and Auto control will be performed in each case to screen for autoantibodies. Adsorption and Elution methods will be used to further investigate for separation and identification of multiple antibodies present in the serum.

### 7.1 SOURCE OF DATA

This study will be conducted among the pregnant females who are registered with the institute for antenatal checkups. OPD Records will be examined thoroughly for history.

The details will be filled as per the performa for each patient.

### 7.2 METHOD OF COLLECTION OF DATA

**Inclusion Criterion:** Pregnant females of all gestation.

**Exclusion Criterion:** Previous history of blood transfusion, history of Rh immunization in present pregnancy, known case of any autoimmune disorders, corticosteroid treatment.

**Duration of Study :**1 Year

**Statistical Analysis:** Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data is normally distributed Comparison of quantitative variables between the study groups will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square

($\chi 2$) test will be performed and fisher exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows

**7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.**

No special investigation or intervention for the purpose of this study only, will be conducted.

**7.4 HAS ETHICAL CLEARENCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?**

Attached herewith.

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# PERFORMA

SR. NO.

NAME:

AGE/SEX:

MRD NO.:

ADDRESS:

PHONE NO.:

DATE OF REG.:

EDUCATION:

OCCUPATION:

MENSTRUAL HISTORY:

MARRIED FOR:

LMP: EDD: GA:

OBSTETRIC HISTORY:

VACCINATION: T1- T2- Tdap-

HIGH RISK:

PAST HISTORY: PREVIOUS TRANSFUSIONS- (YES/NO) IF YES, HOW MANY-

INJ. ANTI-D:

INVESTIGATIONS: ABO Rh BLOOD GROUPING

DAT

IAT

ANTIBODY SCREENING:

ANTIBODY IDENTIFICATION:

ANTIBODY TITRATION:

Signature of Candidate Signature of Supervisor

# INFORMED PATIENT CONSENT

Date: _____

I……..D/o,W/o,S/o…… ……. ……am willing to participate in the study **"PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

| | |
|---|---|
| …… | …… |
| Signature of the Witness | Signature of the patient |
| Name: | |

**Signature of Candidate** **Signature of Supervisor**

# मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____ इस शोधकार्य **"PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

| | |
|---|---|
| _____ | _____ |
| गवाह के हस्ताक्षर | रोगी के हस्ताक्षर/अंगूठे का निशान |
| नाम: | नाम: |

_____

अन्वेषक केहस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

# ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____ ਵਾਸੀ _____

**“PREVALENCE OF CLINICALLY SIGNIFICANT RED CELL ALLOANTIBODIES IN PREGNANT WOMEN”** ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾਮਨੇਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ–ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ